# NATIONAL TOXICOLOGY PROGRAM

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# HEARING

BEFORE THE

SUBCOMMITTEE ON
INVESTIGATIONS AND OVERSIGHT

OF THE

COMMITTEE ON
SCIENCE AND TECHNOLOGY
U.S. HOUSE OF REPRESENTATIVES

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# NATIONAL TOXICOLOGY PROGRAM

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WEDNESDAY, JULY 15, 1981

House of Representatives,
Committee on Science and Technology,
Subcommittee on Investigations and Oversight,
*Washington, D.C.*

The subcommittee met, pursuant to call, at 2 p.m., in room 2325, Rayburn House Office Building, Hon. Albert Gore, Jr., (chairman of the subcommittee) presiding.

Mr. Gore. The subcommittee will come to order.

Since the end of the Second World War, our society has experienced a revolution in its use of potentially toxic substances.

The American Chemical Society now estimates that over 5 million chemicals are currently in existence and 6,000 new chemical structures are identified every week.

Over 55,000 of these chemicals are currently marketed in the United States.

While the vast majority of these substances pose little if any threat to human health, we are all painfully aware of chemicals that have been proven to cause cancer, birth defects, chromosomal changes and other serious human health problems.

Kepone, PCB, Thalidomide, dioxin and countless others have become familiar names over the last few years, as our Nation has begun to understand some of the consequences of the chemical revolution.

Underpinning much of our knowledge and speculation about the human health effects of chemicals is the science of toxicology. We have all seen the commercials and comic strips depicting rats swollen with coffee and dietetic soft drinks, playing with hairdryers that are full of asbestos and laden with handsfull of cigarettes.

As a society we have an infinite capacity to laugh at ourselves, even as we try to perfect the scientific tools on which we must rely.

The realities are that we must rely upon animal toxicology to make the necessary, though imperfect, predictions about toxicity that form the basis of our public health laws. Epidemiology is either too rough or too rare, and obvious ethical considerations block our ability to test chemicals directly upon humans. But test we must.

The challenge is to improve the testing and the research apparatus that supports it. Over the next 2 days the subcommittee will be examining one of the most exciting and innovative experiments to improve the Government's ability to do and to interpret the testing of chemicals.

The national toxicology program was formed in the fall of 1978 to bring together a number of the toxicology programs with the Department of Health, Education, and Welfare in a way that coordinated their activities with each other as well as with other relevant Federal research and regulatory agencies.

The overall aim of the NTP is to develop through research the scientific information needed to protect the public health from damage by exposure to hazardous toxic chemicals.

Nearly 3 years have passed since this ambitious experiment was undertaken, and the time has now come for a first evaluation.

During the next 2 days we will want to answer the following questions:

1. How effective has the organization of the national toxicology program been in bringing together the farflung testing functions of the Government?

2. How successful has the program been in identifying critical problems of research design in toxicology that have inhibited the usefulness of toxicology for regulatory purposes?

3. What is the record of the program in turning toxicology away from routine, and perhaps oversimplified bioassays to more sophisticated and perhaps more predictive tests?

4. To what extent can the experience of the national toxicology program be used as a model for other Federal scientific programs, and particularly for the diverse basic research toxicology programs in other agencies of Government?

5. Can we depend in the future on the national toxicology program to do more of the scientific work necessary before regulatory programs go forward?

And finally, 6, to what extent should Government toxicology programs like the NTP be used as a supplement rather than a substitute for the private testing that is required under a number of Federal regulatory statutes.

These are critical questions that are best addressed now. We must have a Federal toxicology apparatus that is in place and ready to respond to the certain challenges of the future.

As part of this hearing, in the second day, we will also be examining a troubling issue that one ordinarily takes for granted in looking at the scientific programs of Federal agencies, or at any of American science, for that matter.

In the scientific arena, we routinely take for granted that scientists will be permitted to express their scientific opinions freely.

Particularly in the area of scientific regulation, it goes without saying that scientists must be able to express their views to their colleagues as well as to their superiors, without the worry of irrational retribution.

Last year the national toxicology program supported a review of formaldehyde that produced the conclusion that "formaldehyde poses a cancer risk to humans."

Two weeks ago the Department of Labor proposed to fire a senior career scientist essentially for coming to that same conclusion.

No improvements in methodology or scientific testing will matter if the scientists upon whom we are dependent to produce and publish this information are punished for their views whenever an industry's profits are put in jeopardy.

Thus it is important to examine this incident to discover the motivations of the people who would fire Dr. Peter Infante and the consequences of these efforts on our attempts to cope rationally with the toxic substances in our society.

Industry obviously has a key role to play in the development of any rational toxicology program and yet we have no witnesses scheduled from the chemical industry.

I am disappointed that both the Chemical Manufacturers Association and the American Industrial Health Council declined our invitation to testify on these important matters.

I know that they have an active interest in the success of the national toxicology program, and I can only hope that one outcome of these hearings will be an increased incentive for industry and Government to solve these difficult problems together, in open forums, and in ways that speak to the merits of the problems rather than in ways that attempt to muzzle those with whom they disagree.

I would ask unanimous consent that the statement of our distinguished ranking minority member, Mr. Walker, be inserted in the record.

Of course, he will have an opportunity to present it if he wishes.

Mr. GORE. Do either of my colleagues present have an opening statement?

Mr. SHAMANSKY. No, Mr. Chairman. Thank you.

Mr. GORE. Our first witness then is Dr. Gilbert S. Omenn, professor of medicine, University of Washington in Seattle.

Dr. Omenn is the first fellow in science and public policy at the Brookings Institution here in Washington working on issues in science-based environmental regulation.

He is, as I noted, professor of medicine and professor of public health at the University of Washington and is chairman-designate of the department of environmental health and occupational medicine.

During the past 4 years Dr. Omenn served as a Deputy to Frank Press, President Carter's Science and Technology Adviser, and who was Director of the White House Office of Science and Technology Policy.

He has other experience at OMB and we are certainly pleased to have you, Dr. Omenn.

Without objection, the entire text of your written statement will be put into the record and we invite you to proceed with the presentation of any or all of it as you see fit.

## STATEMENT OF DR. GILBERT S. OMENN, PROFESSOR OF MEDICINE, UNIVERSITY OF WASHINGTON, SEATTLE, WASH., AND SCIENCE AND PUBLIC POLICY FELLOW, BROOKINGS INSTITUTION, WASHINGTON, D.C.

Dr. OMENN. Thank you, Mr. Chairman.

I will summarize the testimony.

Mr. Chairman, members of the committee, I am pleased to appear to lead off this important oversight hearing on the complex field of toxicology and on the scientific and bureaucratic innovation that is the national toxicology program.

As you just stated, my personal and professional interests lie in environmental health and genetic research.

My roles in the Carter administration in OSTP and OMB brought me before this committee previously and included participation in a number of initiatives to assess research and risk assessment in areas under review today.

During the past decade hazards and potential hazards to human health from exposures in the many facets of our environment have received much public and congressional attention.

Air pollution, water pollution, pesticides, food additives and contaminants, sewage treatment, chemical wastes, radioactive wastes, chemical byproducts from energy development, and health hazards of cigarette smoking all have been the targets of hearings, laws, regulations, public information campaigns and much litigation.

The absolutely essential base for all of these activities is a reliable, timely, high quality, forward-looking, and open program for scientific analysis of potential hazards.

With more than 40,000 chemicals in common use in this country, many byproducts in the workplace and derivatives occurring from use or disposal, a rational process of priority setting for this scientific effort is essential as well.

As I will indicate, I believe the Federal Government has moved aggressively and effectively to meet those requirements. Doing so has required coordinated action across several scientific disciplines and among a surprising number of Federal agencies.

An especially noteworthy effort at coordination was the Interagency Regulatory Liaison Group, composed of the Administrator of the EPA, the Chairman of the CPSC, the Commissioner of the FDA, and the Administrator of OSHA.

These individuals met regularly and assigned their representatives to working groups in eight areas, some of which we in OSTP assisted, mainly epidemiology, testing guidelines, training and risk assessment.

These agencies often deal with the same chemical hazard from different sources and need the same kinds of scientific expertise and data.

Industrial and consumer groups alike demand consistent approaches from the Government in the identification and characterization of hazards and in the requirements laid upon producers for testing of various kinds of chemicals and other products.

Rational control of exposures depends upon coordinated actions to deal with multiple kinds of sources.

The main disciplines for scientific data gathering are epidemiology, the study of the factors contributing to disease patterns in human populations, and toxicology, the study of the mechanisms of adverse health effects, both in humans and in animals.

Epidemiology and toxicology can be and are being combined to understand and control manmade diseases, just as epidemiology and microbiology were combined to identify and control many infectious diseases. It was clear that more and better data were needed to support the IRLG and that these regulatory agencies did not themselves have adequate resources or expertise to carry out such work.

A determined effort was launched to enlist the broader research community and especially the Federal health research agencies.

A useful and, it turns out, successful mechanism was developed after what had begun, as Tom Grumbly will recall, as something of a shotgun marriage in an experiment in OMB called a zero-based budget review involving the four regulatory agencies and the three major research agencies in chemical toxicology.

The budgets which were most important were the National Cancer Institute, by far the largest and most heterogeneous, the NIEHS, the small budget at NIOSH, plus the research programs of EPA and FDA.

The research agencies agreed to identify more clearly the work which might be most directly related to regulatory agency needs and the four research components of the Department of Health, Education and Welfare, as it was called then, moved ahead with a plan to contribute funds and more importantly active scientific groups to a coordinated research and testing activity which was unveiled on November 15, 1978, as the national toxicology program.

Initial funding was $41 million, all from existing budgets of the National Cancer Institute, the NIEHS, the FDA's National Center for Toxicological Research and NIOSH.

In my opinion, it would have been very desirable to have had some budgetary contribution from EPA, but the EPA did join fully in the executive committee that advises the Director of the program.

We were fortunate that Dr. David Rall, Director of the NIEHS, agreed to take on the additional responsibility of directing NTP. The NIEHS provides an important scientific base for the program, and Dr. Rall has had extensive experience with the full range of Federal agencies, outside interest groups, and industrial organizations focused on these toxicological questions.

We were gratified—and I believe the record will bear out my contention—that the potentially awkward mechanism of the executive committee, including the heads of the four regulatory agencies plus the Directors of the research agencies and of the NIH, proved effective in giving direction on the new chemicals that deserved highest priority for testing and in supporting a steady move from the routine testing of the cancer bioassay program to more investment in assessment of other potential adverse effects and in development of more cost effective and more rapid test protocols.

The progress in the NTP has been described extensively in public documents, including the 1979, 1980 and 1981 annual plans and the NTP newsletters.

I previously skimmed these reports. In preparation for this hearing, I read them very carefully. I must say they merit the reading.

I will make a few comments broadly about toxicological research, especially, Mr. Chairman, since you mentioned the question of the model of NTP in the broader role of basic research programs relevant to toxicology.

In my opinion the field of toxicology is being transformed as a result of the application of powerful techniques of biomedical research.

As occurs so often, the practical yield of research may occur in areas far removed from the direct interest of the basic researchers who investigated processes of cell division, reproduction, neural development, or gene expression.

A prominent example is the work of Dr. Bruce Ames, whom I knew when I worked in the intramural program of the NIH in Bethesda. His work was on the enzyme pathway for biosynthesis of the amino acid histidine in the bacterium Salmonella typhimurium. Many mutants were isolated and characterized. Later in Berkeley in the early days of public attention to environmental hazards, he utilized these bacteria to quantify the effects of chemical mutagens and estimate their potency. The sensitivity, quantifiability and rapidity of the tests were great advantages. Another crucial advantage was the ability to include in the test medium liver extracts from mammals, even humans, that supply enzymes to convert a chemical in ways similar to those occurring when the chemical is ingested by people.

Ames was not then and is not now a toxicologist, but his work is being utilized widely in the rapidly expanding field of toxicology.

Dr. Rall's report of the wide array of biological end points under study by the NTP and especially the NTP report of the four times larger array of relevant toxicological work supported throughout the Department show the breadth of toxicological research and its ability to attract researchers trained in many related disciplines—biochemistry, pharmacology, food sciences, physiology, genetics, chemistry, behavioral sciences, pathology, veterinary medicine, reproduction, teratology, epidemiology, biostatistics, occupational health, chemical engineering and so forth.

It is my recommendation that this process should be left as pluralistic and diversified as possible. I would be happy to address that, if you wish, later.

From the point of view of congressional oversight, I think it will be helpful to describe the task of evaluation of public health hazards from the environment in three steps: identification of a substance as potentially hazardous; characterization of the nature, extent and relative significance of the risk for various segments of the population; and analysis of the potentially effective steps that can be taken or advised to reduce or eliminate the risks.

My colleagues and I in the OSTP published a "Framework for Federal Decisionmaking" that outlined this process of identification, characterization and control of risks.

A copy of that paper, which was published in the Journal of the National Cancer Institute in January 1980, after wide review, is appended to my testimony. I will not summarize it further.

One aspect of testing chemicals that must be emphasized is that not all compounds turn out to be positive when tested for carcinogenicity.

The public reaction and the reaction of many scientists, I fear, is that if you put enough of substances *x*, *y* and *z* into a rat every substance will be found to be carcinogenic. In fact, the results of tests even of samples of chemicals biased to include those which resemble others already known to be positive suggest that only about 20 percent of chemicals are carcinogenic in animals. That means that 80 percent fail to produce such effects, at least within the sensitivity of the tests.

We need to gain confidence that we can deal with what Johnny Carson calls the biggies, and not just the marginal risks. People start saying, "Oh, my God, now it's coffee; last year it was hamburgers; water, air and food are not safe; what's left?" When that happens, we have a problem in credibility and public understanding of this field and its results.

The National Journal recently published an article about saccharin and risk/benefit analysis and decorated the edges of the page with a dozen rats—all supine, all tumorous, all apparently dead; the causes listed were saccharin, air, fried chicken, tea, malted milk, water, blueberry waffles, coffee, lasagna, tobacco, and aphrodisiacs.

It really is not a laughing matter, but it has gotten to the point where fun is all too easily poked.

Characterization of risks must be made in terms that people can comprehend, not only in mathematical expressions derived from quantitative risk assessment methodologies.

For pervasive exposures in which public action is required for protection of the public health, it is essential that broad segments of the population understand and support the proposed actions.

Otherwise Congress will be forced again into the impasse that arose over saccharin, a situation you described well, Mr. Chairman, in your address of April 7, 1981, to the Fourth National Food Policy Conference.

Let me close by directing you to the last part of my testimony which is a suggestion rather parallel to the questions you raised in your own opening remarks of criteria by which the progress to date of the NTP and its program for the next few years might be evaluated.

I have listed eight categories.

The first is priority setting. This is the most important issue and the issue upon which you can probably make the most clear judgment of the interaction of the regulators and the researchers and their capacity to understand each others' problems and capabilities.

Second is efficiency. Has the NTP completed the roster of bioassays already launched under the bioassay program for analyzing the screening of chemicals?

Third, openness. This is of substantial interest to you and your colleagues.

The fourth is coordination and information sharing with other agencies. I think the annual reports, which have brought out study protocols and rosters of activity from other DHHS agencies and this year also from EPA and the Department of Energy, are highly useful.

Fifth is the role of the NTP in advancing the field, in setting up biological assays which are incorporating the latest in carcinogenic criteria and also introducing a host of other biological end points. You can gain opinions from me and others on the scientific side for these more subjective judgments.

Sixth is characterizing the risk in terms I just indicated, especially of opening up the "black box" of statistical analysis and mathematical extrapolations so people can understand the variables and the reasons for the uncertainties that may remain.

Seventh, in responding to emergencies, has the NTP been helpful to the regulatory agencies? Has it maintained its independence and not had its resources diverted?

Finally, as to the size of the program, with the current budgetary constraints, how can one plan for the size and appropriate growth for the NTP? How will its role be defined in relation to the many other agencies performing toxicological work and, as the bioassay roster inherited from the NCI is completed, should the NTP maintain a certain number of fixed high priority chemicals or should the Nation expect that the manufacturers will take on a larger role and the NTP can be more selective in dealing with those of special scientific value?

I know most of these questions will be addressed during today's testimony.

I believe the chemical selection process has gone well and that appropriate principles for selection have been adopted. The Executive Committee and the Board of Scientific Counselors have been effective participants in the decisionmaking processes for the program. Dr. Rall and Dr. Moore have provided strong leadership.

The expansion of the bioassays to include many other biological effects is laudable and efficient. And the broad array of work on new kinds of tests is very promising.

My own impressions of the accomplishments and management of the NTP are highly favorable.

I look forward to hearing the testimony of others today and to participating in your inquiries.

Even more, I look forward to a productive Federal and national program in the many aspects of toxicology over the next decade.

[The prepared statement of Mr. Omenn follows:]

## STATEMENT OF DR. GILBERT S. OMENN

Mr. Chairman and members of the Committee, I am pleased to appear today to lead off your important oversight hearing on the complex field of toxicology and on the scientific and bureaucratic innovation known as the National Toxicology Program. Your invitation cited both my personal interests in environmental health and genetics research and my participation in the previous Administration in a number of initiatives to enhance research and risk assessment in areas under review today. From June, 1977 until March, 1980, I was an Associate Director of the Office of Science and Technology Policy (OSTP), under Dr. Frank Press, President Carter's Science and Technology Adviser. Mr responsibilities were health, policy, the full range of life sciences research, and certain institutional and personnel issues. From March 1980 until January 20, 1981, I served as an Associate Director of the Office of Management and Budget (OMB), including responsibilities for the budgets of the programs under review today. In real life, I am a physician and reseacher in Seattle, a professor of medicine at the University of Washington. On July 1, I returned to Washington, D.C. as the first Science and Public Policy Fellow at the Brookings Institution. I am writing about environmental health issues and preparing to accept the challenge of chairing the Department of Environmental Health and Occupational Medicine at the University of Washington early next year.

### ORIGINS OF THE NATIONAL TOXICOLOGY PROGRAM

During the past decade, hazards and potential hazards to human health from exposures in the many facets of our environment have received much public and Congressional attention. Air pollution, water pollution, pesticides, food additives and contaminants, sewage treatment, chemical wastes, radioactive wastes, chemical by-products from energy development, and health hazards of cigarette smoking have been the targets of hearings, laws, regulations, public information campaigns, and much litigation. The absolutely essential base for all of these activities is a reliable, timely, high-quality, forward-looking, and open program for scientific analyses of potential hazards. With more than 40,000 chemicals in common use in this country and with many byproducts in the workplace and derivatives occurring from use or disposal, a rational process of priority-setting in the scientific effort is essential, as well. I believe the Federal government has moved aggressively and effectively to meet these requirements.

Doing so has required coordinated action across several scientific disciplines and among a surprising number of Federal agencies. When the Council on Environmental Quality established a Toxic Substances Strategy Committee in response to the Toxic Substances Control Act of 1976, 22 agencies were represented. Such an unwieldy group needed 3 years to produce its report. An especially noteworthy effort at coordination was the Interagency Regulatory Liaison Group (IRLG), composed of the Administrator of the EPA, the Chairman of the CPSC, the Commissioner of the FDA, and the Administrator of OSHA. These individuals met regularly and assigned their representatives to working groups in eight areas, including four in which our Office of Science and Technology Policy in the White House was asked to assist: epidemiology, testing guidelines, training, and risk assessment. Their agencies often deal with the same chemical hazard from different sources and need the same kinds of scientific expertise and data. Industrial and consumer groups alike demand consistent approaches from the Government in the identification and characterization of hazards and in the requirements laid upon producers for testing of various kinds of chemicals and other products. Rational control of exposures depends upon coordinated actions to deal with multiple kinds of sources.

The main disciplines for scientific data gathering are epidemiology, the study of the factors contributing to disease patterns in human populations, and toxicology, the study of the mechanisms of adverse health effects, both in humans and in animals. Epidemiology and toxicology can be and are being combined to understand and control man-made diseases, just as epidemiology and microbiology were combined to identify and control many infectious diseases. It was clear that more and better data were needed to support the IRLG and that these regulatory agencies did not themselves have adequate resources or expertise to carry out such work. A determined effort was launched to enlist the broader research community and especially the Federal health research agencies. Something of a shotgun marriage was attempted when OMB in 1978 chose toxic substances research as a topic for a zero-based budget review across agencies. The agencies included were the four regulatory agencies listed above, of which only EPA and FDA had significant research budgets, plus the National Cancer Institute (NCI), the National Institute of Environmental Health Sciences (NIEHS), and the National Institute of Occupational Safety and Health (NIOSH). The review served a valuable function in educating representatives from the different agencies about the detailed programs of basic and applied research in their counterpart agencies and in identifying some areas of common interest and activity. However, a divergence of approach became apparent when the regulatory agencies sought to have funds already in the NCI budget diverted from basic work on carcinogenesis to be used for routine testing of chemicals for which data were needed in the regulatory process. This divergence reflects a general problem of the past decade, that we so often have been caught in a crisis situation about one or another chemical exposure without sufficient scientific information or experience to be confident about taking responsible actions.

A useful and, it turns out, successful resolution was achieved. The research agencies agreed to identify more clearly the work which might be most directly related to regulatory agency needs, and four research components of the Department of Health, Education, and Welfare moved ahead with a plan to contribute funds and, more importantly, active scientific groups to a coordinated research and testing activity which was unveiled on November 15, 1978, as the National Toxicology Program. Initial funding was $41 million, all from existing budgets of the National Cancer Institute (its bioassay program), the NIEHS, the FDA's National Center for Toxicological Research in Pine Bluff, Arkansas, and NIOSH. In my opinion, it would have been very desirable to have had some budgetary contribution from EPA, but the EPA did join fully in the Executive Committee that advises the Director of the Program.

We were fortunate that Dr. David Rall, Director of the NIEHS, agreed to take on the additional responsibiltiy of directing NTP. The NIEHS provides an important scientific case for the program, and Dr. Rall has had extensive experience with the full range of Federal agencies, outside interest groups, and industrial organizations focused on these toxicological questions. We were gratified, as well, that the potentially awkward mechanism of the Executive Committee, including the heads of the four regulatory agencies plus the directors of the research agencies and of the NIH, proved effective in giving direction to the NTP, in reaching agreement on the new chemicals of mutual interest that deserved highest priority for testing, and in supporting a steady move from the routine testing of the cancer bioassay program to more investment in assessment of other potential adverse effects and in development of more cost-effective and more rapid test protocols. The progress in the NTP has been described extensively in public documents, including the 1979, 1980, and 1981 Annual Plans and the NTP newsletters.

Its budget has grown from $41 to $67 to $70 million, so that new work could be supported both from increases in the total budget and from funds freed up as the five-year cycles of planning, testing, and analyzing data on individual chemicals were completed. In some cases substitutions were made in the chemical to be tested, or chemicals were withdrawn from study because manufacturers had ceased production.

## TOXICOLOGICAL RESEARCH

The field of toxicology is being transformed as a result of the application of powerful techniques of biomedical research. As occurs so often, the practical yield of research may occur in areas far removed from the direct interest of the basic researchers who investigated processes of cell division, reproduction, neural development, or gene expression. A prominent example is the work of Dr. Bruce Ames, who worked in the Intramural Program of the NIH for many years on the enzyme pathway for biosynthesis of the amino acid histidine in the bacterium *Salmonella typhimurium*. Many mutants were isolated and characterized. Later in Berkeley in the early days of public attention to environmental sources of mutagens and carcinogens, Ames utilized the well-characterized *Salmonella* system to test chemicals for their capability and potency to cause mutations. The sensitivity, quantifiability, and rapidity of the tests were great advantages. Another crucial advantage was the ability to include in the test medium liver extracts from mammals, even humans, that supply enzymes to convert a chemical in ways similar to those occurring when the chemical is ingested by people. Ames was not then and is not now a "toxicologist," but his work is being utilized widely in the rapidly expanding field of toxicology.

Dr. Rall's report of the wide array of biological end-points under study by the NTP and especially the NTP report of the four-times larger array of relevant toxicological work supported throughout the Department of Health and Human Services show the breadth of toxicological research and its ability to attract researchers trained in many related disciplines—biochemistry, pharmacology, food sciences, physiology, genetics, chemistry, behavorial sciences, pathology, veterinary medicine, reproduction, teratology, epidemiology, biostatistics, occupational health, chemical engineering, and so forth. Given the severe shortage of well-trained fully-credentialed toxicologists and the difficulty of Federal agencies and academia in competing with industry for such scientists, it is desirable that well-trained scientists from cognate fields be attracted to multidisciplinary research in toxicology. As noted in a report published by the Conservation Foundation in 1978, "Training Scientists for Future Toxic Substances Problems," there is a shortage of training opportunities for people interested in this broad field. Training grant support combined with research funds to attract scientists from cognate fields and to build multidisciplinary research and training programs is sorely needed.

From the point of view of Congressional oversight, I think it will be helpful to describe the task of evaluation of public health hazards from the environment in three steps:

Identification of a substance as potentially hazardous;

Characterization of the nature, extent, and relative significance of the risk for various segments of the population; and

Analysis of the potentially effective steps that can be taken or advised to reduce or eliminate the risks.

My colleagues and I in the OSTP published a "Framework for Federal Decision-making" that outlined this process of identification, characterization, and control of risks. A copy of that paper, which was published in the Journal of the National Cancer Institute in January, 1980 after wide review, is appended to my testimony. Special recognition was given to the NTP as an important innovation in priority setting and coordination of testing to serve the several regulatory agencies and the larger public.

One aspect of testing chemicals that must be emphasized is that not all compounds turn out to be positive when tested for carcinogenicity. The public reaction and the reaction of many scientists, I fear, is that if you put enough of substances X,Y, and Q into a rat every substance will be found to be carcinogenic. In fact, the results of tests even of samples of chemicals biased to include those which resemble others already known to be positive suggest that only 10 to 20 percent of chemicals are carcinogenic in animals. That means that 80 percent or more fail to produce such effects, at least within the sensitivity of the tests. We cannot be certain that these chemicals are perfectly safe, but we can be reassured and focus our control efforts on the others. We need to gain confidence that we can deal with what Johnny Carson calls the "biggies," and not just the marginal risks. When people start saying, "Oh, my God, now it's coffee; last year it was hamburgers; water, air, and food are not safe; what's left?" The National Journal recently published an article about saccharin and risk-benefit analysis and decorated the edges of the page with a dozen rats—all supine, all tumorous, all apparently dead; the causes listed were saccharin, air, fried chicken, tea, malted milk, water, blueberry waffles, coffee, lasagna, tobacco, and aphrodisiacs!

Characterization of risks must be made in terms that people can comprehend, not only in mathematical expressions derived from quantitative risk assessment methodologies. As a physician I am reminded regularly that different people have very different responses to specific risks and to risk-taking. For pervasive exposures in which public action is required for protection of the public health, it is essential that broad segments of the population understand and support the proposed actions. Otherwise, Congress will be force again into the impasse that arose over saccharin, a situation you described well, Mr. Chairman, in you address of April 7, 1981, to the Fourth National Food Policy Conference.

## EVALUATION OF THE NTP

Criteria by which to evaluate the NTP progress to date and its program for the next few years may be organized as follows:

1. *Priority-Setting.*—Has the Program achieved a rational basis for selection of chemicals for testing, incorporating the recommendations of the various regulatory agencies? Will data from these test cycles be available in time for Congressionally-mandated schedules of revision of criteria documents for specific pollutants, for example? Have chemicals with multi-media exposures received special attention?

2. *Efficiency.*—Has the NTP managed to complete the roster of bioassays already launched under the NCI Bioassay Program or deleted any no longer requiring test? Has the pre-chronic screening of chemicals been a useful basis for decisions on which chemicals receive the full investment of several years, 600 rats, and half a million dollars for bioassay?

3. *Openness.*—Has the NTP shared plans and results at various stages in a way that attracts helpful information from all kinds of knowledgeable sources? Has the information released by NTP been adequately reviewed and properly characterized? Has the NTP been viewed by professional industrial, consumer, and environmental groups as both cautious and responsible in protecting the public health?

4. *Coordination and Information-Sharing.*—Has the NTP, through its massive Annual Reports, helped the other DHHS agencies, as well as EPA, DOE, and perhaps other Federal agencies, to improve planning for their toxicological research programs?

5. *Advancing the Field.*—Has the NTP made an adequate transition within the budget to more and more investment on potentially better and more rapid tests and a broader array of biological effects than just tumor production? Has the NTP, or the basic research agencies whose studies NTP summarizes, begun to identify chemcials with risk-reducing properties, such as anti-oxidants, which may protect against certain carcinogenic compounds in the stomach or intestinal tract, or other chemicals which might enhance genetic repair systems?

6. *Characterization of Risk.*—Has the NTP, alone or working in concert with risk assessment units at the various agencies, advanced the field of risk assessment by application to new and better data of specific mathematical models of extrapolation to low doses in humans? Has the risk assessment process 'opened up the black box' of statistical analysis and mathematical extrapolations—by characterizing carefully the nature of the health effects produced, the possible predisposing conditions in animals or humans studied by toxicological or epidemiological methods, the possible species differences in metabolism and disposition of the chemical, the extent of exposures, and the probability that certain segments of the human population will be at higher risk than others? Has the NTP stimulated the basic research agencies, including the NIEHS, to investigate the mechanisms of the health effects observed, so that we may not have to rely indefinitely on statistical associations without clear knowledge of the action of the chemical?

7. *Responsiveness in Emergencies.*—Has the NTP developed information which proved useful in emergency responses to unanticipated exposures to a specific tested chemical or a chemical whose effects could be deduced as a result of studies of related chemicals? Was the NTP able to buttress the short-term efforts of first-line

agencies in testing newly-encountered hazards? Did the NTP divert too many resources to such emergency situations?

*8. Size of the Program.*—With the overall budgetary constraints and with the severe personnel constraints within the NIH and Public Health Service, how much further growth in budget or personnel is warranted for the NTP? How will its role be defined in relation to the many other agencies performing toxicological work even within DHHS? As the large roster of chemicals already under bioassay is completed, should the NTP maintain a certain fixed number of high-priority chemicals under test? Or should the implementation of the Toxic Substances Control Act of EPA place more responsibility for testing of chemicals on the manufacturers, with the NTP serving a far more selective role, dealing with complicated mechanisms or conflicting results on chemicals in wide use or likely to become widely used?

I expect that most of these questions will be addressed during today's testimony. I believe the chemical selection process has gone well and that appropriate principles for selection have been adopted. The Executive Committee and the Board of Scientific Counselors have been effective participants in the decision-making processes for the program and Dr. Rall and Dr. Moore have provided strong leadership. The completion of the Bioassay Program roster, I suspect, has been arduous. The expansion of the bioassays to include many other biological effects is laudable and efficient. And the broad array of work on new kinds of tests is very promising. My own impressions of the accomplishments and management of the NTP are highly favorable.

I look forward to hearing the testimony of others today and to participating in your inquiries. Even more, I look forward to a productive Federal and national program in the many aspects of toxicology over the next decade.

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# Identification, Characterization, and Control of Potential Human Carcinogens: A Framework for Federal Decision-Making[1]

**D. R. Calkins,[2] R. L. Dixon,[3] C. R. Gerber,[2] D. Zarin,[2] *and* G. S. Omenn[2,4,5]**

**ABSTRACT—A two-stage process is proposed for a uniform framework for Federal agency decisions regarding the identification, characterization, and control of potential human carcinogens. Stage I would include the identification, through epidemiologic and/or laboratory studies, of chemicals that represent a potential carcinogenic risk and the characterization of that risk. Stage II would encompass the actual regulatory decision-making process regarding control of potential carcinogens. Stage I relies predominantly on scientific activity and judgment. Centralized management could enhance the efficiency and effectiveness of this process. The new National Toxicology Program may be able to perform this function. Stage II judgments are social and political. Centralization of stage II decision-making is not possible under current law.—JNCI 64: 169-176, 1980.**

The American Chemical Society estimates that more than 4 million chemicals are in existence, and about 6,000 new ones are identified every week (*1*). Approximately 44,000 of these chemicals are believed to be in common use in the United States. Their use has in many ways improved the quality of our lives. At the same time, however, many have been found to be potentially hazardous to humans. One of the challenges facing modern society is to determine how best to reconcile these potential hazards with the potential benefits to be obtained from the uses of these chemicals. Numerous laws and regulations address this challenge. Some require the banning of a chemical shown to pose potential hazards to human health; others require controlled use in order to reduce hazards.

Presently, the leading public concern about exposures to chemicals is the risk of cancer. Consequently, considerable effort in both the public and private sectors is being devoted to determination of what chemicals are or might be carcinogenic. Many Federal agencies are charged with identifying, controlling, and/or regulating potentially carcinogenic compounds. Unfortunately, the various laws enacted to protect the public from such hazards have resulted in both gaps and areas of overlap in agency responsibilities, as was documented recently in a report prepared for the Toxic Substances Strategy Committee (TSSC) (*2*). The Environmental Protection Agency (EPA) (*3*), the Consumer Product Safety Commission (CPSC) (*4*), the Occupational Safety and Health Administration (OSHA) (*5*), and the Food and Drug Administration (FDA) (*6*) independently have developed policies for assessment and regulation of these chemicals. Several interagency activities[6] are under way in an effort to achieve a more consistent Federal approach to this problem. Although it is impossible under current law to achieve a single Federal system to control carcinogens, it is feasible for the Federal Government (and its various agencies) to adopt a consistent approach for deciding what chemicals pose a carcinogenic risk to humans, for characterizing the nature and extent of that risk, and for evaluating alternative Federal (agency) actions.

In the approach recommended in this paper, the decision-making process is divided into two stages. In stage I, research would be performed and evaluated to characterize the cancer risk attributable to human exposure to a particular chemical or class of chemicals. To the extent feasible, this risk would be expressed in comparative and quantitative terms describing the various elements of uncertainty.

In stage II, the responsible agencies would assess the

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[5] Previous versions of this paper were circulated for individual and agency review on October 20, 1978, and February 1, 1979. We are grateful to the following individuals, who commented on those earlier drafts: Dr. Robert Abelson, Yale University; Mr. Roy E. Albert, Environmental Protection Agency (EPA); Dr. James Beall, EPA; Dr. Edward Burger, Georgetown University; Mr. Douglas Costle, EPA; Mr. Lynn Daft, Domestic Policy Staff, Executive Office of the President; Mr. Amitai Etzioni, Brookings Institution; Dr. Joseph F. Fraumeni, Jr., National Cancer Institute; Dr. Sidney Galler, Department of Commerce; Dr. P. J. Gehring, Dow Chemical Company; Dr. Robert Gordon, National Institutes of Health; Dr. Philip Handler, National Academy of Sciences; Dr. Jeff Harris, Massachusetts Institute of Technology (M.I.T.); Mr. D. Mark Hegsted, Department of Agriculture; Dr. David Jackson, Case Western Reserve University; Dr. Henry Jacoby, M.I.T.; Ms. Susan King, Consumer Product Safety Commission; Dr. Caro Luhrs, consultant; Dr. Norton Nelson, New York University; Dr. David P. Rall, NIEHS; Mrs. Dorothy Rice, National Center for Health Statistics; Dr. Joseph Rodricks, Food and Drug Administration; Dr. David Rutstein, Harvard University; Dr. Carl Shy, University of North Carolina; Dr. Eugene Skolnikoff, M.I.T.; Dr. Robert Solow, M.I.T.; Dr. Arthur C. Upton, National Cancer Institute; and Dr. James Whittenberger, Harvard University.

[6] Ongoing Federal efforts at interagency coordination in this area include the Regulatory Council, the National Toxicology Program (NTP), the Interagency Regulatory Liaison Group (IRLG) and its various working groups [including one on risk assessment, whose report has previously been published in this Journal (7)], the TSSC, the Department of Health, Education, and Welfare Committee to Coordinate Toxicology and Related Programs, the Clearinghouse on Environmental Carcinogens, the Interagency Toxic Substances Data Committee, and the Interagency Collaborative Group on Environmental Carcinogenesis.

significance of that risk, the benefits (if any) to be derived from the chemical, and the appropriate means (if any) for limiting its use. To the extent allowed by law, stage II decision-making would reflect assessment of all relevant benefits and costs as well as their societal distribution. In regulatory decisions, benefits result from a reduction in risk of cancers attributed to the chemical regulated, whereas costs are generated by loss of economic value (adjusted for the effect of substitutes) and by investments in control technology. As in stage I, both the analysis and the resultant regulatory decision would take into account uncertainties in available data.

The purpose of this paper is to elaborate on certain aspects of the two-stage framework, particularly the scientific processes relevant to stage I, and to share with a broad scientific audience recommendations made to the responsible Federal agencies and interagency committees.

Two points deserve emphasis before we proceed further. First, we do not insist that the boundaries between stage I and stage II be defined rigidly or that the two processes be performed by unrelated agencies. The information needs of stage II decision-makers must be known to stage I research scientists. The judgmental elements underlying stage I conclusions regarding carcinogenic risk must be understood by stage II regulators. Thus the need for effective communication and feedback is clear.

Second, the focus of this paper on stage I should not infer that we place any less importance upon stage II or upon the need for a more nearly consistent approach to stage II decision-making. Rather, this focus reflects the particular interests of this Office and our view that the issues involved in stage I analysis are more amenable to consensus at the present time than are the issues in stage II, for which the existing law often limits the ability of an individual agency to alter its decision-making approach.

## IDENTIFICATION OF POTENTIAL CARCINOGENS

Basic to control of carcinogens is the ability to identify chemicals that are potentially carcinogenic in humans. Several kinds of information can be useful. For chemicals already in use, human epidemiologic studies sometimes offer the first indication of carcinogenicity. Laboratory data may be needed to buttress such findings, because isolation of the effect of a single chemical on humans is often difficult. For new chemicals, reliance only on human experience obviously would be inappropriate and unwise; the long latency period of many carcinogenic effects would require waiting as long as 5-40 years to learn if a chemical presents a risk to humans. Thus both for chemicals already in the environment and for new ones, laboratory methods must be utilized to screen for carcinogenic potential. Laboratory tests currently include both animal bioassays and short-term in vitro methods. Each of these testing approaches has value in establishing the potential carcinogenicity of a chemical for humans. It is important that policy-makers understand the advantages and the limitations of these methods.

### Epidemiologic Data

For an epidemiologic study to produce evidence of carcinogenicity (on the assumption that the chemical under study is in fact a carcinogen), two criteria must be met: 1) Exposure to the chemical in the study population must be at a sufficient level and/or sustained over a sufficient period of time to result in a measurable (and statistically significant) increase in cancer incidence. 2) A control population must be identified that is comparable to the study (exposed) population in all relevant factors except exposure to the chemical under study. Both of these points deserve further discussion.

The need for high and/or prolonged exposure may be explained on both a biologic and statistical basis. In general, the lower the exposure level and the briefer the period of exposure, the lower will be the incidence of chemically induced cancer in a given population. For an increased cancer incidence to be apparent under these conditions, larger study and control populations would be required.

For a given level and duration of exposure, two additional factors will modify the size of study and control populations necessary to achieve statistically significant results: 1) the potency of the suspected carcinogen under study and 2) the base-line incidence rate of the relevant cancer in the population. Simply stated, the more potent the carcinogen and the less common the cancer, the greater will be the likelihood of achieving statistically significant differences in cancer incidence in a given study. For example, epidemiologic studies have revealed an association between exposure to vinyl chloride and the development of angiosarcoma of the liver. This association could be demonstrated by assessment of a relatively small population because the carcinogen (vinyl chloride) is moderately potent and the cancer (angiosarcoma) is rare. Had the carcinogen been less potent or the cancer more common, a larger population would have been necessary. For the extreme case (i.e., a very weak carcinogen and a very common tumor), such a study would not be feasible.

The criterion of comparability between study and control populations is often difficult to achieve. Even if the two populations have comparable demographic variables, one cannot be certain that they have comparable aspects of life-style and environment that affect cancer risk. A complete analysis of all factors known or thought to affect cancer risk is not feasible. Even if it were, reporting of personal behavior and environmental exposure cannot be relied upon to be accurate. Furthermore, unknown factors may create a cancer risk differential in the two populations.

Problems in monitoring exposure levels and assuring comparability between study and control populations are substantial in a prospective epidemiologic study. They are compounded in retrospective studies. Exposure data are often not recorded. Personal recollections of exposure and/or life-style data are often fragmentary and potentially inaccurate. Although retrospective studies can usually be performed more expeditiously, they are less likely to yield reliable results.

Despite these difficulties, a positive finding in a well-conducted epidemiologic study can be viewed as strong evidence that a chemical poses a carcinogenic risk to humans. Alternatively, a negative finding is not nearly so meaningful because of the many factors that could obscure a positive association between exposure to a chemical and an increase in cancer incidence.

We must emphasize that the report of a negative epidemiologic finding is incomplete without an analysis of the sensitivity of the study (i.e., the lowest increase in cancer incidence that could have been detected by the methodology employed). Such analyses might help resolve the confusion that may arise when a chemical appears to be a carcinogen in animal tests but not in human epidemiologic studies. Such analyses could serve also as a quantitative benchmark in extrapolation from animal data to human exposure by identification of an upper confidence limit on the magnitude of cancer risk associated with a given level of exposure in humans.

Epidemiologic studies occasionally suggest a positive association between a chemical and cancer when previous animal tests of the same chemical have been negative. Such discordance may be attributable to species differences in susceptibility to the chemical or to one or more confounding variables in the study population. A diligent search for such variables should be made in an attempt to explain the discordant findings.

### Recommendations

*Federal guidelines should be established for the design, conduct, and interpretation of epidemiologic studies for use in regulatory decision-making along the lines discussed above.*

*Positive evidence of carcinogenicity from well-designed epidemiologic studies should be accepted as a basis for regulatory action, though further testing is appropriate when contradictory evidence is available from other human and/or animal studies.*

## Tests on Animals

Numerous recommendations have been made regarding the design, conduct, and interpretation of animal bioassays (7-9). A consensus is emerging on a number of issues, three of which we highlight here:

*Extrapolation from animal studies to humans.*—Virtually every chemical shown to be carcinogenic in humans has also been shown to be carcinogenic in other mammals (arsenic and benzene are the major apparent exceptions). Although proof is impossible that the converse is true, it is prudent to view a positive test result in a carefully designed and well-conducted mammalian study as evidence of potential human carcinogenicity.

Some researchers have suggested that the results of high-dose testing in animals are not relevant to humans in whom exposure levels are lower. We reject this view. High-dose testing is a necessary feature of laboratory bioassays because of the limitations of time and available test animals. The finding of cancer in animals given only high doses does not imply that such doses would be required in humans, particularly in view of the longer exposure periods existing for humans. Animal dose levels should, however, be limited so that they do not induce overt toxicity unrelated to carcinogenesis. Toxic doses may alter metabolic processes, resulting in a unique carcinogenic effect not present at lower levels of exposure.

*Interpretation of conflicting data.*—Divergent results may occur either among tests on different species or among different tests on the same species. Any test that is poorly designed or poorly conducted must be viewed as less meaningful than one that is done properly. If appropriate testing procedures are followed, however, it is generally sound to let positive results supersede negative results in tests involving different species, taking into account the frequent occurrence of interspecies variation and the inability to determine which species experience is most relevant to that of humans. In the interpretation of divergent results from separate studies within the same species, all of the data should be considered.

Interpretation of apparently conflicting data can be facilitated by careful attention to both biologic and statistical criteria for a "positive" outcome. Biologic features of a positive outcome include appropriate dose-response and time-dose relationships and absence of signs of noncarcinogenic toxicity from high doses. When evidence of carcinogenicity is found only at toxic doses, such evidence should be rejected as demonstrating carcinogenic potential for humans if it seems reasonable to conclude that the toxic effect noted could have created a unique susceptibility to cancer in the animal population not characteristic of human exposures.

Statistical criteria for conclusion that a study is positive are discussed in the section "Statistical Analysis."

*Assessment of promoters or cocarcinogens.*—Some animal species show unusually high incidence rates for particular cancers under normal conditons. It has been argued that a chemical that only increases an already high cancer rate should be viewed as a promoter or cocarcinogen (rather than a direct-acting carcinogen) and should be treated differently in the regulatory process. Although such an argument may have merit, current routine laboratory methods do not generally allow one to distinguish promoters or cocarcinogens from direct-acting carcinogens. Until such methodology becomes available, we propose that a chemical that increases the cancer incidence rate in laboratory animals be viewed as a potential human carcinogen and treated accordingly by regulatory agencies.

### *Recommendation*

*All agencies supporting animal tests or using such test results for regulatory decision-making should adopt uniform testing guidelines and/or protocols.*

The value of animal test data would be enhanced if uniform criteria can be established regarding the performance and interpretation of such tests. Such criteria should define *a*) appropriate species, strains, and sexes of laboratory animals; *b*) minimum number of animals in control and experimental groups; *c*) types of controls needed; *d*) appropriate routes of exposure; *e*) appropriate range and level of doses; *f*) purity of test chemicals; *g*) adequacy of animal housing and care; *h*) minimum observations times; *i*) types of histologic lesions considered; and *j*) proper statistical evaluation of the results. It is essential to recognize that bad science is of no value in the regulatory process.

## Short-Term Screening Tests

As the number of chemicals being developed and coming into use has grown, a need has arisen to find more expeditious and less costly means of testing for carcinogenicity. Efforts have been focused to date on in vitro tests of mutagenicity, inasmuch as the capacity of a chemical to cause mutations in certain in vitro test systems has been shown to correlate well with its capacity to induce cancer in animals or humans.

The best studied in vitro test is the Ames-salmonella assay. One recent evaluation (*10, 11*) of this test with 283 chemicals (175 known animal carcinogens and 108 noncarcinogens) demonstrated a sensitivity of 90% and a specificity of 87%. Many of the false negatives (10% of known carcinogens) were judged to be chemicals that require metabolic activation in vivo to exert a mutagenic effect; others may act via hormones or other mechanisms unique to mammals. Alternatively, the false positives (13% of noncarcinogens) have sometimes been shown to be weakly carcinogenic or contaminated by mutagenic impurities; in other instances they may have been tested inadequately in bioassays.

Despite rapid improvement in in vitro testing procedures, today no single in vitro test is either 100% sensitive or 100% specific in identifying potential carcinogens. As a result, the predictive value positive of a single in vitro test can often be quite low.[7] The predictive value positive can be increased by the use of a battery of tests (if the battery is interpreted as positive when all tests are positive and if the results of the various tests are statistically independent). However, even a positive outcome on a battery of tests cannot be interpreted as absolutely indicative of carcinogenic potential. It thus seems prudent to treat in vitro tests (either individually or as a battery) as screening tests only.

### *Recommendation*

*Federal guidelines should be established for the appropriate use of short-term in vitro tests in screening for potential human carcinogens.*

At the present time, the short-term in vitro test may be most useful in the establishment of priorities for animal testing or for epidemiologic studies of existing chemicals and in the premarket testing of new chemicals. Despite the high false-positive rate and low predictive value positive, companies are likely to forego further development of most chemicals found to be positive in screening tests and to accelerate further testing and development of chemicals found to be negative in screening tests.

## Statistical Analysis

The testing methods discussed above cannot provide a simple "yes" or "no" answer regarding the potential for human carcinogenesis. Rather, test results offer a probability that such a potential exists. In evaluating such a test, one examines the data and then performs a statistical test of the null hypothesis—that no difference exists between the control and experimental groups in cancer (or mutation) rate. If there is, indeed, a difference, the statistical test will provide a probability (expressed as *P*-value) that such a difference could have been observed due to chance alone. The statement that a test is "significant at a *P*-value of less than 0.05" means that, if the null hypothesis were true (i.e., if no actual difference existed between the control and the experimental groups), then the probability would be less than 5% that such a difference could have been observed due to chance alone.

By convention, scientists often choose a *P*-value of less than 0.05 as "statistical proof" that the null hypothesis is false. However, any given *P*-value on a single test must be viewed in the context of other available data. For example, if a sufficient number of tests is performed, predictably a "statistically significant" difference will be found in at least one test regardless of the *P*-value chosen, simply as a result of random variation.

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[7] Percent predictive value positive = true positives/(true positives + false positives)×100. The predictive value positive is dependent on the sensitivity and specificity of the test and on the prevalence of the test characteristic in the population under study. A test that is both 95% sensitive and 95% specific, performed in a population with a prevalence rate of 5%, will yield a predictive value positive of only 50%. The Ames-salmonella assay with a specificity of 87% would yield an even smaller predictive value positive in such a population. In general, the more specific the assay or the greater the prevalence of the characteristic in the population under study, the greater will be the predictive value positive.

###### Recommendation

*The outcome of any single test should be viewed as potentially the result of chance variation. Regulatory action should proceed only when the preponderance of all available evidence points toward carcinogenicity.*

The establishment of a specific *P*-value (e.g., 0.05) as a standard for "statistical proof" represents an oversimplification and a misapplication of statistical analysis. One should always consider both the biologic and statistical features of a positive outcome in analyzing test results.

## CHARACTERIZATION OF HUMAN RISK

The determination that a particular chemical might be carcinogenic in humans is only the first step in the characterization of potential human risk. Several other items enter into the risk equation: potency, nature of exposure, and variations in individual susceptibility. Data on each of these factors must be sought and some quantitative estimate of overall risk (and uncertainty) must be made if regulatory agencies are to establish prudent controls over the use of potential carcinogens.

### Potency

Direct epidemiologic information permitting dose-response calculations for carcinogenicity in human populations is seldom obtainable. Quantified estimation of human carcinogenic potency generally requires extrapolation from animal data to relevant human exposures. Such an extrapolation presents several problems. Testing performed in several species frequently reveals interspecies variations in tumor response. Thus a range of dose-response estimates may be derived from animal studies. More importantly, most animal data, as already noted, are obtained from testing at high exposure levels. Therefore, it is necessary to extrapolate downward from these levels to the lower levels to which humans may be exposed. Several alternative models have been developed for this purpose, but there is no general agreement as to which one provides the most reliable estimate of potency at low levels.

Despite these problems, we believe that a quantitative estimate of human carcinogenic potency can and should be incorporated into any overall estimate of carcinogenic risk. Although precise quantification of human carcinogenic potency may not be possible, few would argue that a chemical that requires only a few micrograms to produce cancer in a given test animal should not be viewed with more concern than another chemical requiring several grams to produce a similar type and incidence of cancer in the same animal (all other factors being equal).

###### Recommendation

*A quantitative estimate of potency should be used as one element in the characterization of overall human risk from a potential carcinogen.*

All appropriate human epidemiologic data that are available should be used to estimate the tumor incidence expected from various human exposure levels. When no such data are available, carcinogenic potency should be estimated from animal studies, noting the range of values obtained in various animal systems and the dose-response model used for extrapolation to low doses. That the estimate is derived from animal, not human, data should also be clearly acknowledged.

An assessment of the relative potency of potential human carcinogens will aid agencies in the establishment of regulatory priorities and in the selection of appropriate regulatory action. The finding of relatively low potency should not, itself, however, suggest that a chemical does not warrant agency attention. A potential human carcinogen of even low potency may present a substantial risk if individual exposures are high or if large numbers of people are exposed. In addition, a chemical of relatively low potency and low carcinogenic risk may be worthy of regulatory action if the benefits to be derived from its use are judged to be small.

### Nature of Exposure

Characterization of risk must also take into account the nature of human exposure. Several questions must be asked: What are the sources of the chemical? Does the chemical encountered from each of these sources undergo any environmental alteration? By what routes and at what rates do humans come in contact with the chemical? How do these different routes and rates affect the risk? How many individuals are so exposed? Although considerable strides have been made in recent years in the identification of potential human carcinogens, little work has been done to define the nature and extent of actual human exposures to these chemicals.

###### Recommendation

*Characterization of risk should include consideration of the nature of human exposure to potential carcinogens. Appropriate research should be undertaken to determine sources, prevalence, and levels of exposure, as well as any environmental alteration of the chemical under consideration.*

To the extent allowed by law, regulatory decisionmakers should consider these exposure factors. In some instances, assessment of such data might lead to a decision (where the law permits) to eliminate only a single source of human exposure if this source is found to account for the bulk of overall risk. Alternatively, if only a small number of individuals is exposed, specific action might be taken to protect them from a particular carcinogenic risk.

### Individual Susceptibility

As in the case of exposure, several factors must be considered in defining individual susceptibility to a potential carcinogen. For example, particular populations or individuals may have their cancer risk altered by age, sex, genetic predisposition, or exposure to other chemicals (*12*). These sources of variation in individual susceptibility have received less research attention than their importance warrants, especially in light of the efforts by regulatory agencies to protect what are thought to be the most susceptible groups or individuals in the general population (*13*).

#### Recommendation

*Characterization of risk should consider individual susceptibility.*

Additional research is needed to define metabolic alteration of potential carcinogens as well as populations at special risk. Where the law permits, regulatory action might seek selective reduction of exposure levels in susceptible populations.

### Combining Risk Factors

In evaluating overall human cancer risk from a particular chemical, one could construct an equation describing the relative impact of each factor described above (potency, exposure, and susceptibility). Risk could be expressed in terms of increased cancer cases or cancer deaths expected as a result of exposure to that chemical. As noted above, current data do not often allow a precise estimate of potency, exposure, or susceptibility. What value should be entered into the equation for each of these factors to obtain an accurate characterization of overall risk? A commonly used approach is to select a risk estimate from the high end of the range for each term in the equation. This practice results in an extreme estimate of human risk. Another approach is to use the most likely value for each term, including confidence intervals that express the precision of each estimate; this approach generates a risk estimate that has a greater probability of being accurate and seems to us to be the more logical.

Although several mathematical models for estimating human risks have been produced, we are not prepared to recommend any particular one. We expect that experts from the various research and regulatory agencies can come to some agreement about the relevant variables, the weight to be accorded each, and the best means of evaluating the uncertainty in current data.

#### Recommendation

*Characterization of the overall risk for humans in quantitative terms should be the final step in the stage I process. The characterization should use the most likely value for each term in the risk equation and should include an appropriate representation of uncertainty.*

Stage II decision-makers should be given the most accurate risk estimate possible and should be informed clearly about uncertainties in existing scientific data and their impact upon the risk estimate. Judgments about the necessary overall margin of safety should be left to stage II, in which costs and benefits attributed to various potential regulatory actions (and resultant decreases in exposures) can be considered.

## DETERMINATION OF PRIORITIES FOR STAGE I REVIEW

Because of the large number of chemicals now on the market and the steady flow of new ones, priorities must be set both for testing (identification) and characterization of risk. For testing, highest priority should be given to chemicals for which preliminary analysis suggests that regulation may be indicated but for which existing data are too uncertain to justify immediate action. These chemicals would generally include ones that have a high potential for carcinogenicity (based upon structural similarity to known carcinogens or the results of short-term in vitro tests) but have had only minimal prior testing, ones that offer substantial consumer benefits, and ones that do not have readily available means of control.

For characterization of risk, priorities again should be resolved by the likelihood of regulatory action. Complete characterization of risk is mandatory for any chemical that has been clearly defined as a potential human carcinogen and should be done first for those with greatest apparent potency and/or greatest potential human exposure.

#### Recommendation

*Highest priority for testing should be given to chemicals for which regulatory action is likely but not yet feasible because of uncertainty in current data. Priority for characterization of risk should be given to chemicals already identified as potential human carcinogens, especially those for which potency and/or exposure are thought to be high.*

## ORGANIZATIONAL APPROACHES TO STAGE I

We have indicated the necessary steps in the identification and characterization of potential human carcinogens as well as the need to set priorities for these activities. Until recently, each agency of the Federal Government has performed these functions in an independent and uncoordinated fashion. There has been no mechanism to assure early attention to chemicals of greatest carcinogenic potential, efficient allocation of analytic resources, or consistent assessment of human risk.

Several recent initiatives by this Administration, namely, the IRLG, the Regulatory Council, and the NTP, provide the foundation on which to build a better organizational and management structure for the stage I functions.

Priority setting and coordination of testing for the identification of potential human carcinogens ideally should be done by a single group, with input from all relevant agencies. The newly formed NTP is a logical body to assume these functions. It brings together on its executive committee the heads of the FDA, EPA, CPSC, OSHA, the National Institutes of Health, the National Cancer Institute, the National Institute of Environmental Health Sciences, and the National Institute of Occupational Safety and Health (NIOSH)—the "users" (regulatory agencies) as well as the "doers" (research agencies). The initial mandate of the NTP is to coordinate and improve priority-setting and allocation of resources for laboratory research. In the near future, the role of the NTP could be expanded to encompass epidemiologic studies.

Impartial scientific judgment is crucial for evaluation of data and characterization of risk because of the uncertainties in the data or, at times, the lack of data. At present, each regulatory agency peforms its own characterization of risk utilizing its own standards and methodologies. Both the EPA and FDA perform characterization of risk internally. For OSHA, the characterization of risk is done by NIOSH, an external but closely affilitated agency. Much remains to be learned from these continuing agency-specific arrangements.

We believe that greater coordination of the characterization of the risk process is possible. Our minimum proposal is that uniform procedures for characterization of risk should be followed by all regulatory agencies. The recent work of the IRLG in this area can serve as a starting point for interagency agreement on characterization of risk. In instances in which a particular chemical may be of interest to more than one agency, we believe that centralized management of characterization of risk by an organization such as the NTP would be desirable.

### *Recommendation*

*All stage I activities should be coordinated eventually by an expanded NTP, managed by an executive committee composed of all relevant research and regulatory agencies. The NTP should establish priorities for and coordinate funding of laboratory testing and epidemiologic studies for all agencies. The IRLG or the NTP should also establish uniform procedures for characterization of risk. Characterization of risk activities relevant to more than a single agency should be coordinated under the aegis of the NTP.*

To assure that stage I conclusions are clearly understood in stage II, we recommend that a member of the regulatory staff be assigned to the group that performs the characterization of risk function. In addition, we suggest that the final regulatory decision be reviewed by an independent, scientifically qualified group to assure that the regulatory decision is consistent with the scientific conclusions resulting from stage I. Under the Clean Air Act Amendments of 1977, the EPA is establishing this practice with its Scientific Advisory Board.

## CONCLUDING REMARKS

The Federal Government can and should establish a uniform framework for decisions regarding identification, characterization, and control of potential human carcinogens. The decision-making process proposed here has two parts. Stage I includes the identification of chemicals that pose a potential carcinogenic risk to humans and a characterization, in quantitative terms, of the nature of that risk and the degree of certainty of current estimates. We view this stage as dependent primarily on scientific activity and scientific judgments. We propose that stage I activities would benefit from centralized management by an expanded NTP as that program evolves over the next few years.

Stage II is the process of choosing among various regulatory options. Although centralization of stage II processes is not possible under current law, more effective coordination is feasible and desirable. A broad review of stage II procedures has been initiated by the Regulatory Council, which was established by President Carter in November 1978. In addition, a Regulatory Analysis Review Group, established in 1977, assesses the overall benefits to society and the cost impact of selected major regulatory actions. These two actions should improve the coordination and consistency of regulatory decision-making.

We are confident that adoption of a uniform decision-making framework as proposed here can improve the quality and efficiency of regulatory decision-making. By facilitation of public understanding of this process, such a framework could enhance public confidence in the Federal Government's efforts to assure the health and safety of the American people.

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Mr. **Gore**. Thank you very much for being with us and leading off this hearing.

I would like to inform my colleagues that because of the number of witnesses and the lateness of our start, we are going to observe the 5-minute rule today.

I would like to call first on Mr. Shamansky.

Mr. **Shamansky**. Dr. Omenn, in your opinion should the national toxicology program be a separate agency directly funded by the Congress, and, if not, should any changes be made in the way the NTP is funded such as identifying its funding as a line item in the OMB budget or possibly strengthening the current memorandum of understanding of the funding mechanism?

How do you, based on your experience, feel would be the best way?

Dr. **Omenn**. My judgment is that the program has operated well in its present, rather amorphous and clearly cooperative kind of arrangement.

It was not easy to structure it the way it is.

I would be pleased to hear from Dr. Rall and also from the other agency heads whose moneys are involved, their own judgment of the most practical arrangement for this program.

I should, however, react not just from the OSTP point of view, but from my experience in OMB I was astonished when I came to OSTP and then when I took on the responsibilities for the budget, that such a large budget as the $3.6 billion in NIH was dealt with in the executive as a whole.

It was studied from the point of view of the mechanisms of funding, training, individual grants, centers, contracts, intramural program, but not institute by institute.

That is to say that the executive did not review the NIH budget in the same way that the Congress does in its appropriations.

Mr. **Shamansky**. Doesn't that raise the question though, based on your OMB experience, if it weren't given a line item, it might just sort of disappear one day?

Dr. **Omenn**. I think that is not likely.

The same question could be asked about the entire program of the NIH and what Congress has done is to very fastidiously appropriate moneys for the programs within institutes and for the functions within and across institutes.

I have every confidence that the Congress will continue to provide that kind of oversight for the NTP, as will the Assistant Secretary, and the OMB, since agencies outside NIH have a stake in the NTP. Therefore, it would be desirable to identify the total budget of the NTP and be able to examine its needs in relationship to the total program of the Public Health Service. Some consolidation of the budget components of the NTP may be a timely and efficient move at this point in the development of the program.

Mr. **Shamansky**. Given that, in your view can the NTP effectively plan a multiyear research program, given the apparent uncertainty and potential variability of funding levels present in the currently used NTP memorandum of understanding, regarding its funding mechanism?

Dr. **Omenn**. Congressman Shamansky, I can only say that we have this problem throughout the entire research enterprise. This committee knows only too well that for the kinds of problems addressed in scientific research and development the annual budget cycle is simply insufficient and not effective from an efficiency and cost point of view. It is a great frustration in planning. It is a great frustration in trying to be prudent with the taxpayers' moneys.

Mr. **Shamansky**. What you are saying is that it is the best we have and you have to try?

Dr. **Omenn**. Yes. I think annual budgeting is what we are living with. Until you are willing to address that on a broader scale, we are stuck with the problem for the NTP.

Mr. **Shamansky**. Do you think they should be given the authority to direct research or should its role be that of a coordinator of resources offered to the program?

Dr. **Omenn**. I am a firm believer in the NIH program and its intramural and extramural reliance upon individual investigators.

I think in general we had half decade of the early seventies too much directed research.

There is a great risk. I indicated in my testimony that we cannot predict with confidence where important new developments will come from that will possibly revolutionize toxicological testing.

The program on which we now rely the most, the cancer bioassay, is too large an investment per chemical, so tedious, so demanding of animal and human resources, that while we rely on it now and we should and must do so, we should expect the broad array of research to somehow yield something better in the near or medium term future.

I think that directed research in this area is adequate at this present time. I think Dr. Rall and Dr. Moore have gotten cooperation from the other agencies. They can speak for themselves.

My impression is that the cooperation has been good; that there is adequate joint planning in the selection process for chemicals, that it has worked out well.

The criteria are understandable and have been applied. I would be reluctant to suggest that more resources go to the program at this time or that the resources be directed in a way that would infringe upon the activities of the other agencies, including the regular programs of the NIEHS.

Mr. **Shamansky**. Thank you, Doctor.

Thank you, Mr. Chairman.

Mr. **Gore**. The gentleman's time has expired.

Mr. Walker?

Mr. **Walker**. Thank you, Mr. Chairman. First, I would like to ask unanimous consent that I be permitted to submit an opening statement at that point appropriate in the record following your opening statement.

Mr. **Gore**. We already did that for you.

Mr. **Walker**. Thank you, Mr. Chairman.

Second, rather than questioning Dr. Omenn right off here, I would like to respond to something which was contained, I believe, in your opening statement because it relates to something that will be coming up later on in the hearings.

That, Mr. Chairman, relates to the case of Dr. Peter Infante who was mentioned earlier in your statement.

I must say it bothers me to see this case being tried in the press and before the Congress in both forums.

The *Infante* case appears to have been misstated. The issue is not one of freedom of scientists to seek the truth.

The issue is whether senior Government employees are free to use their official positions to resist official policies with which they disagree.

Dr. Infante is charged with insubordination. He was free to express his personal views, but the charge is that he represented his personal viewpoint as the agency's viewpoint.

That is a substantially different kind of thing than to say that his scientific judgment is being questioned.

In any case, he faces an administrative proceeding with numerous procedural safeguards. He will also have access to the courts to protect his rights.

While I am not a lawyer, I question whether it is proper to prejudice an ongoing case by interjecting ourselves into it at such a stage.

It is well established law that each administration has the right to establish policy. If senior fellow employees can't support those policies it does not follow they should be permitted to conduct guerrilla warfare against such policies.

To substitute personal opinion for public policy creates a rather unmanageable situation and perhaps more important it creates a chilling effect.

It is a chilling effect that is antimanagement and may also be to some extent antiscience.

I say that because the policy that government pursues tends to be based upon a large body of scientific evidence, not the judgment of one particular scientist.

If we allow one particular scientist to impose his viewpoint as public policy which has not been totally cleared, we could end up with a situation where science itself comes under question.

I think that that is something that we need to reflect upon.

Managers must be free to conduct personnel actions on the merits of the case. I think that the merits of the Infante case are what we should be concerned with.

It is also what we should be concerned are ongoing as the case moves forward.

Thank you, Mr. Chairman.

I have no questions.

Mr. GORE. Mr. Volkmer?

Mr. VOLKMER. No questions.

Mr. GORE. Dr. Omenn, do you think that membership on the Executive Committee of the NTP should be broadened to include other federal agencies interested in toxicology research and testing like the Department of Energy, DOD, the Department of Agriculture, et cetera?

Dr. OMENN. I think the present Executive Committee is adequately large. It is a good balance of research and regulatory agencies.

To make it larger would bring it into the realm that I indicated before of the 10, 15, 20 agency committees on which I personally participated and which were so unwieldy as to be quite impractical.

I think there are other interagency mechanisms for identifying the programs in research of those other agencies; their programs are modest compared to that marshaled by the NTP and the Department of HHS.

In this current annual report, the NTP has already gotten cooperation from DOE and EPA in reporting their programs. I expect that information sharing might be expanded in future years to some other agencies.

I am sure Dave Rall could recount for you the many committees on which he already serves in which NOAA, Agriculture, many other agencies are represented.

I think this is a very special program. To make it larger would have not much likelihood of gain and substantial risk.

Mr. Gore. My impressions of the NTP at the outset of these hearings, without having had the benefit of listening to all of our witnesses yet, is that it is an innovative experiment to come up with a new approach that has been in large measure successful but can be improved.

It is an experiment that raises questions and raises a challenge for the Congress too.

It does not appear anywhere as a line item, and that means that for it to be held accountable we have to maybe come up with novel approaches here in the Congress too.

To whom, in your opinion, should the NTP be accountable—accountable for its expenditures of funds if it remains both a partly coordinated and partly managed research program?

Dr. Omenn. In the executive it is clear that the program is accountable to the Assistant Secretary of Health in DHHS.

That line of reporting is clear. It is clear also that its budget and program is under review in the OMB.

The reason I point to OMB is that the array of agencies interested, including OSHA and EPA and CPSC outside of the Department, have their own watchdogs and advocates within the OMB staff and are interested to see that their regulatory needs are addressed and that the research programs to which they contribute somehow mesh with the program of the NTP and the much larger program, including all that basic work in the HHS agencies.

From Congress point of view, this matter of coordination and oversight is an important issue which we have talked about before with other members of the committee, Chairman Fuqua, for example. When I was in OSTP that was discussed.

In general, oversight in the Congress, it appears to me, has been given over to the appropriations process. Budgetmaking, as in the executive, is such a dominant aspect of an agency's life that most of the program review is gained through the detailed review of budget requests and the granting of that line.

This hearing is a test for the agency's program and for the committee to see if oversight on the program with information about the budget, but without the budget in the balance, can be as instructive.

I suspect it can be. I think you have examples in which that is the case.

Mr. Gore. Identify for me, if you can, the amount of increase in funding for the NTP since it was established. It does not appear as a line item, but if you bring together all of the dedicated amounts, what is the growth in funding?

Dr. **Omenn**. My understanding is as follows: The program was begun in fiscal year 1979 with budget authority transferred from the four participating agencies of $41 million.

I learned by reading the fiscal year 1981 report from the NTP that in fact $47.7 million was outlaid which means there must have been some shifts before the year was completed in what actually could be supported. The program must have been expanded either for the work that was agreed to or for certain emergencies.

The budget for fiscal 1980 was $66.7 million and the estimate for 1981, I believe, is $70.1 million. Those numbers are identified in the reports from NTP.

It clearly indicates which amount of money comes from which of the four contributing agencies, those pie diagrams you have probably received.

Mr. **Gore**. I would ask unanimous consent to put into the record at this point a document prepared by our staff that outlines the growth in funding from $47.6 million in 1979 to $73.2 million in the coming year. The latter figure is an estimate.

[The information follows:]

National Toxicology Program
by Contributing Agency

(Dollars in Thousands)

| | 1979 Actual | 1980 Actual | 1981 Estimate | 1982 Estimate |
|---|---|---|---|---|
| National Cancer Institute. . | $25,812 | $43,495 | $45,686 | $49,034 |
| National Institute of Environmental Health Sciences. . . . . . . . . | 10,177 | 11,307 | 12,526 | 14,780 |
| Food and Drug Administration/National Center for Toxicological Research . . | 7,000 | 6,300 | 6,140 | 6,922 |
| Centers for Disease Control/National Institute for Occupational Safety and Health . . . . . . . . | 4,700 | 4,608 | 2,500 | 2,500 |
| Total . . . . . . . . . . | 47,689 | 65,710 | 66,852 | 73,236 |

Note: 1981 and 1982 based on President Reagan's budget.

Mr. **Gore**. These figures will show that the lion's share of the increase has been in the bioassay program at the National Cancer Institute, doubling from 25 to almost 50.

Do you think further growth in that particular aspect of the NTP program over at NCI is warranted at the same continued rate?

Dr. **Omenn**. I think no further growth in the bioassay program is warranted.

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Mr. GORE. No further growth in that program is warranted? Why?

Dr. OMENN. I think the NCI and NTP have invested substantial amounts in the bioassay program. A tremendous amount of valuable information has been obtained.

It has been difficult to generate as much scientific interest as NIH traditionally expects from its scientists and workers because so much of the bioassay program is routine testing of yet another chemical.

The reason that that growth had to be so substantial between 1979 and 1980, as I understand it, is that NTP was inheriting with very full cooperation from NCI a very large roster on the order of 225 chemicals.

As you know, the cycle for testing a single chemical is about 5 years from the time it is planned; the chemical is obtained in adequate purity to give to animals, various preliminary studies are done, 2 or 3 years of lifetime observations in rodents are done, and the reports are analyzed. That is a 5-year cycle.

This year they are completing work that began 4 or 5 years ago. They still have a substantial number.

That work has been pushed. Progress is good. Meanwhile the regulators sat down with the research agencies and figured out—as you have seen it in the documents, or if you haven't, Dr. Rall will present it—the half dozen or eight criteria for selection of chemicals nominated by the agencies for testing: scientific interest, a chemical in wide use and not enough information about it, or likely to be put into wide use, chemical relatives are known to be or thought to be hazardous.

In order to make good on that kind of effort at nominating and selecting new chemicals, there had to be moneys to start some new chemicals in the bioassay program.

Furthermore, the cost of doing business has gone up in this field, as in most others.

I think the increase between 1979 and 1980 was fully warranted. I think the balance which has been maintained, in which a fourth or a third of the moneys are going into development and validation of new tests, is pretty good considering the backlog which must be dealt with.

Mr. GORE. Do you believe the NTP contributing agencies, such as NCI, should eventually transfer those designated resources to NIEHS in order that NTP become a more tightly managed research program, or is that a bad idea?

Dr. OMENN. I don't think it is a bad idea. It may require a little more fleshing-out than I have heard. I am not in the government now, so I don't know the current details. Also I think the relationship between NIEHS and NTP in terms of their budgets needs to be made clear.

You have both Dr. DeVita and Dr. Rall here. It is their budgets. They know those programs far better than I do.

I think that clarity in how the money is going to be spent and more importantly clarity in the objectives of the program should be secured whether they are in different pots or put into a single pot.

Mr. GORE. I would be delighted to yield at any point to any of my colleagues if you have any other questions.

I just have a few more.

In your view, how aggressively has the NTP pursued a highly prioritized chemical selection process, and what should be the highest priority for chemical testing in your opinion?

Dr. OMENN. The answer to the first part of the question is, I think they have pursued it aggressively. They have involved appropriate people from the agencies to be served, the regulators, and knowledgeable people from their own research agencies.

They have had a substantial open public process with public meetings and invitations to manufacturers and public interest groups and other groups.

They have elicited plenty of comment. My impression is that the list is sound, of high priority and not just a list of individual chemicals, but of groups of chemicals such as the benzidine dye study and some of the others which hopefully will yield a range of results from which predictive value can be gained.

Just exactly what should be the highest priority? I haven't thought about it carefully that way. I think there is a matrix of criteria that come together in that list of criteria for the chemical selection process. Obviously a chemical which is in wide use with substantial dose exposures to humans, which is alleged to have adverse effects and on which the data are conflicting should have very high, if not the highest, priority for urgent testing by the most modern methods.

Mr. GORE. As we pointed out, this program really is the basis for so many other actions taken by the Federal Government, and the need for regulators for more accurate quantitative risk assessment has become increasingly apparent.

Do you believe that the NTP can make a contribution with a quantitative risk assessment? What is your opinion of the NTP's bioassay protocol, with only two exposure levels in regard to this need?

Also, how valuable have the results of the bioassay tests been in developing an accurate quantitative risk assessment?

Dr. OMENN. My impression is that the NTP is grappling appropriately with the problems of risk assessment and quantitative risk assessment. There is a good deal of risk assessment to be done that in my opinion is better termed "characterization," because so much of it is qualitative and has to do with the explanation and description of the uncertainties and the methods, the differences across species, the basis for extrapolating to humans.

I think some of the really nasty controversy about quantitative risk assessment has to do with the notion that different people use it for different purposes, and in its present embryonic form it needs more development than rote application.

I think it is getting that and going to get more of that from the NTP. The data that are coming out are being fed into a data management system where information previously collected from many other sources concurrently obtained by industry or other groups, or obtained from epidemiological studies, are put together with the results of the whole array of biological end points in the assay programs of the NTP. That is exactly what ought to be done.

Whether a specific number can be placed on the risk assessment for humans is something that is a researchable question, but it will have to be accompanied with description of the uncertainty in statistical terms and in good plain English. Furthermore, the underlying assumptions and extrapolations must be explained.

Let me say that studies of the kind NTP performs or sponsors, in which the same test systems are used for many different compounds, can build an array of data where you have quantitative information in comparable systems in the same labs, with interlab comparisons. From these results, one can build quantitative estimates for the relative potency of compounds in those test systems.

That progress must be accompanied by much better information than we presently have on exposures. Exposures is the neglected side of this biological effects and exposures picture.

The nature of the exposure, the sources, the extent in terms of population exposed, and the time course, all of that is essential in order to describe adequately and to plan for control measures that may be warranted for a particular class or group of chemicals.

Mr. GORE. My specific question is about the bioassay protocol and it is my final question.

I do want to try to get a response to this aspect of it.

As I understand, with the bioassay protocol that is now followed, there are only two exposure levels. They go to what the immediate toxic dose is; they find out that quantity; then they cut it in half and take those two quantities.

Is that optimum or adequate or should it be modified, reconsidered, or reevaluated? Do you think that it is OK?

Dr. OMENN. I think it is OK assuming that is the way it is done. It is OK for present purposes. I think that you have to be careful not to push beyond the tolerable level of the animal and you want to have an adequate dose to get an effect if there is one to be had.

Obviously if you are studying only one chemical in a research lab, you would like to know everything about it.

You would like to know the dose response curve and lots of other features. You cannot do that in a screening program. Screening is the intention of the NTP.

Screening is different from research. Testing is different from research. Trying to do some of both is a tremendous challenge.

Mr. GORE. But you cannot use that kind of protocol for a dose response curve?

Dr. OMENN. If you don't have more than two points, you can't have a dose response curve at least not without additional assumptions.

Mr. GORE. If none of my colleagues——

Dr. OMENN. I must add to that. In some cases, there may be many other data on related compounds or on the same compound from other studies which could be put together with the two points of screening studies obtained by the NTP.

Mr. GORE. Thank you very much for getting us off to a good start. We appreciate it very much.

Mr. GORE. I would like to call now a panel of two witnesses, Dr. Edward N. Brandt, Jr., Assistant Secretary for Health with the Department of Health and Human Services, and Dr. David Rall, Director of the National Toxicology Program as well as Director of the National Institute of Environmental Health Sciences.

## STATEMENTS OF DR. EDWARD N. BRANDT, JR., ASSISTANT SECRETARY FOR HEALTH, DEPARTMENT OF HEALTH AND HUMAN SERVICES; AND DR. DAVID RALL, DIRECTOR, NATIONAL TOXICOLOGY PROGRAM AND DIRECTOR, NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES, ACCOMPANIED BY DR. JOHN MOORE, DEPUTY DIRECTOR, NATIONAL TOXICOLOGY PROGRAM

Mr. **Rall.** Thank you, Mr. Chairman.

Mr. **Gore.** Gentlemen, your prepared statements will be in the record. Please proceed as you see fit, Dr. Brandt.

Dr. **Brandt.** Thank you, sir.

Mr. Chairman, members of the committee, I appreciate the opportunity to appear here today to discuss the National Toxicology Program, its progress, and the tasks which lie before it.

In this century we have seen considerable progress in the control or eradication of the infectious diseases which in past centuries have erupted and swept across whole cities and nations.

Advances in the control of infectious diseases have been some of medicine's and public health's proudest achievements.

During the past 30 years we have seen extraordinary developments in clinical medicine. Although this work is certainly not completed, we can all take pride in these achievements because to a large degree they rest on three decades of investment in, and encouragement of, biomedical science as an important and appropriate activity of the Federal Government.

During the same period that saw these remarkable developments in the biomedical sciences, we began to learn that toxic substances in our environment can cause or increase the likelihood of contracting disease.

At the same time, we have learned that some chemicals can adversely affect our developing young, sometimes before birth, sometimes after.

The earliest Federal attempts to deal with problems of chemical toxicity took place in the pharmaceutical and food additive industries where extensive safety testing was required by the Federal Food, Drug and Cosmetic Act.

This testing, however, was primarily for acute effects and did not address the consequences of long term exposure to chemical agents.

In the early 1960's the National Cancer Institute began a program intended to determine whether particular substances could cause cancer in experimental animals as a result of long-term, low-level exposure.

These early efforts were the antecedent of NCI's carcinogenesis bioassay program, a program of long-term administration of chemicals to laboratory animals, which remains the principal method for determining whether substances are carcinogenic.

But, while such laboratory testing contributed greatly to the development of the knowledge base regarding the carcinogenic potential of chemicals, it did not attempt to predict for toxic effects other than cancer.

Prior to this time and continuing into the seventies, advances were being made in other areas of research that eventually would permit a more comprehensive approach to the problems of chemical toxicity.

Information was being developed that indicated many environmental chemical agents interfere with cell replication and thus might have an adverse effect on genetic inheritance.

Moreover, information was developed that indicates that a compound which is mutagenic also may be carcinogenic.

During this time the climate of public and congressional concern about the safety of chemicals brought about intensified testing efforts within various research and regulatory agencies.

Within the Public Health Service, the National Cancer Institute ran a cancer bioassay program; the National Institute of Environmental Health Sciences did a modest amount of long-term chemical testing and developed short-term tests for a variety of disease end points; the National Center for Toxicological Research was testing a number of chemicals of interest to the Food and Drug Administration and was doing research in teratology and chemical disposition; and the National Institute for Occupational Safety and Health of the Centers for Disease Control was studying the toxicity of chemicals found in the workplace.

These agencies were often looking at the same compounds, but for different reasons.

It was against this backdrop of scientific, regulatory, and congressional concern that the Department established in November 1978 the national toxicology program.

So that regulatory concerns would be joined with the program, the Occupational Safety and Health Administration, the Environmental Protection Agency, and the Consumer Product Safety Commission were asked to participate in the NTP.

From the outset, the NTP has been guided by the following goals:

To broaden toxicological characterization of those chemicals being tested.

To increase the rate of chemical testing, within the limits of available resources.

To develop and begin to validate a series of protocols more appropriate for regulatory needs.

It is important to remember that the NTP was and is a new concept in interagency relationships and thus could be expected to evolve unique approaches to the complex set of science management tasks which it was assigned.

We now see several patterns of management and control among the contributing agencies.

For example, the NIH component, which represents 85 percent of the NTP budget, is directly controlled by NTP. However, the CDC and FDA components have evolved into more loosely coordinated programs while maintaining full participation in overall NTP activities.

The NTP mechanism also has been involved in several special efforts I would like to mention.

The NTP has tested or is continuing to test 70 of the over 200 chemicals found at the Love Canal site.

Staff members have played an active role in the interagency effort to evaluate the health effects of agent orange and dioxins.

NTP, in collaboration with the Interagency Regulatory Liaison Group, recently held a 3-day conference on phthalates, a class of chemicals used widely to make plastics. This was a joint effort with the Chemical Manufacturers Association and I am pleased to see this information sharing between our program and industry.

One important initiative is a project to evaluate benzidine, a human carcinogen, and benzidine-based dyes.

It is important to point out that this important study was designed through the cooperation of scientists from all the NTP member agencies.

We have tried to make the NTP have as open a process as possible. We have published the NTP annual plan and solicited comments on it. We have held open meetings to receive comments on the NTP. We also have provided the draft bioassay reports for review and comment.

I believe that the national toxicology program has much improved our ability to effectively coordinate the complex toxicological research the Department conducts.

At the same time I recognize that there are areas of needed improvement.

As you know, within the Department, the Office of the Assistant Secretary for Management and Budget has conducted a management review of the program. That review was completed in 1980.

The study team reported it found indications of initial success by the NTP in moving toward each of its goals.

For example, they reported significant increases in short-term testing. These tests focus on toxicities other than carcinogenicity, are completed in months, and cost in the tens of thousands of dollars significantly less than long-term bioassays; development of three new standard screening methodologies; broadening of the protocols in several long-term tests so that short-term tests are performed concurrently with the long-term ones; development of an open process to nominate chemicals for testing.

I intend to use that study as part of my assessment of the NTP and where it should go from here.

I want to make it clear that I strongly support the NTP and the concept underlying it.

My review, however, is focusing on the following: appropriate budgetary levels for the NTP; the organization and permanence of the NTP structure; an assessment of the program's usefulness to regulatory agencies; and the appropriate balance of testing that should exist between the NTP and industry and better ways to improve communication between the program and industry.

While as scientists and public health practitioners we feel we do not know enough, it is likewise true that in the last decade we have learned a great deal about how toxic chemicals adversely affect our health.

Given the long latency period of much disease, we have found that many of the tools we have developed to combat the infectious diseases and which have proven effective in those areas have to be adapted.

The fact that these chemicals may only increase the incidence of an existing disease, rather than cause a new and unique one, makes particularly difficult the task of associating a given disease, or increase in incidence, with a particular chemical exposure.

What we need now are better tools for determining which chemicals, and combinations of chemicals, are hazardous and which are not, and how these chemicals act to express their effects.

The coordination and cooperation in toxicological research that has been brought about by the NTP is helping us to find more quickly those better tools.

The NTP has also formed an important bridge between the scientist and the regulator so that knowledge is shared and mutual problems discussed.

I believe the NTP represents an effective and imaginative approach to interagency planning and cooperative research. It is also my belief that it is moving to meet the goals set for it in 1978.

At this point I would like to call on Dr. Rall to discuss the NTP in more detail.

Thank you.

Mr. Gore. Welcome, Dr. Rall. We look forward to hearing from you.

Dr. Rall. May I first introduce on my right Dr. John Moore, deputy director of NTP?

I am pleased to be here and discuss my perception of the progress of NTP over the last 2½ years.

I think it is important that we have made significant strides in improving the long-term animal bioassay. Formerly this only gave a yes or no answer as to whether a chemical caused cancer in animals.

We are developing an integrated approach to toxicological testing using an expanded experimental design.

The ultimate goal is to measure the full toxicological potential for each chemical studied.

Bioassays have been expanded to include studies of genetic damage, reproductive effects, and damage to kidney, liver, lungs, and the immune and nervous systems.

We also do hematological investigations and urinalysis. About 70 percent of the 40 chemical starts in fiscal year 1980 included such special studies. All chemical starts developed in fiscal year 1981 have an expanded design.

A major improvement is that by 1982 we expect to study the distribution, metabolism, and excretion of most of the chemicals we are testing. This is important because most chemicals are metabolized or chemically altered in the body to different and frequently more toxic compounds.

Further, it aids in the selection of proper dosage and dose regimen for the long-term study.

The design of each bioassay is supervised by key scientific staff of the NTP. The protocol for each bioassay is individually tailored to gain as much useful information on the effects of the chemical as possible.

An NTP committee monitors these studies as they progress and reviews and approves the draft report before it goes to our board of scientific counselors' peer review Committee for independent scientific scrutiny.

We want the final result not to be a mere statement that a chemical does or does not cause cancer in animals, but a complete toxicological profile, one which evaluates previously published studies found in our literature search, one which looks at a wide variety of possible toxic effects and one which can be published in the literature so that the scientific community can have access to this information without having to write the Government for a copy of the report.

While this process goes on, we make the results of prechronic tests and status reports available to interested investigators, regulatory agencies, et cetera, so that others who are looking at the same problem can have access to what we are doing without waiting the full 3 or 4 years this process usually takes.

In addition to making significant improvements in the long-term bioassay, NTP is developing new tests and protocols that are needed to meet future toxicological testing requirements, particularly in fertility and neurobehavior. We are developing new short-term in vitro tests to allow more rapid screening of chemicals or perhaps more importantly, mixtures of chemicals for cancer potential—short of the 2-year rodent bioassay—and to test for other toxic effects.

In the development of new short-term test systems, we want to assure that the test is adequately sensitive, minimizes false positives and false negatives, can be standardized and produces results that are reproducible from laboratory to laboratory, and finally yields results that are meaningful in terms of human health.

That is a tall order. If it seems like we are not moving very rapidly, I think you can see why.

Another NTP effort is the chemical mutagenesis testing program, which has two primary goals: To determine how different types of chemicals affect genetic structure and to correlate mutagenicity results with those for carcinogenicity.

We routinely evaluate the mutagenicity potential for all chemicals being bioassayed. So far almost 900 chemicals have been or are being examined for mutagenicity.

I might add the results of these tests are rapidly published in our NTP technical bulletin.

In other stages of development are programs to develop short-term tests for neurobehavioral toxicity, impaired kidney and pulmonary function, and a teratogenesis prescreen. We expect to validate a fertility and reproduction assessment assay during the coming 2 years.

We have also begun an interlaboratory study of an immunotoxicity test battery and have let two contracts for a short-term lung adenoma test for carcinogenicity that, if validated, should produce results in 6 months instead of 2½ years.

In carrying out this task, the NTP is guided by its executive committee, composed of the heads of the major health regulatory and research agencies.

The committee advises me on research and testing needs; helps nominate, evaluate, and select chemicals for testing and sets priorities for those selected; and oversees program planning and balance of NTP testing and test methods development programs.

I would like to discuss two recent research initiatives with which the executive committee has been involved. The first is an assessment of the toxicology of derivatives of benzidine, a human carcinogen.

As the use of benzidine itself as a commercially important dye has decreased, attention has focused on the substitution of chemical derivatives of benzidine.

It now appears that these derivatives are metabolized in the body and free benzidine is excreted in the urine. Many of these benzidine derivatives are in use today but only a few of them have been tested for carcinogenicity.

It is important to determine which of these compounds are converted in the human body to free benzidine, which are mutagenic in short-term tests and which should be tested for carcinogenicity in long-term studies.

All of the NTP agencies are collaborating in this effort.

Another research initiative involves the phthalates. These interesting compounds—about 1.3 billion pounds a year are marketed in the United States—are used primarily to soften plastics.

Phthalates were discovered to be rather ubiquitous in the environment about 1970. Of particular concern is the fact that phthalates were found in hospital blood bags and in the blood used for transfusions.

Since this class of compounds had not been tested for long-term toxicity, NTP in the mid-1970's initiated a phthalates testing program. NTP testing now has shown that certain of the phthalates are carcinogenic and certain may have other important toxicity effects.

NTP, the IRLG, and the Chemical Manufacturers Association organized an international symposium in early June dealing with the problems of phthalates toxicity.

NTP will serve as the international clearinghouse for the development of research programs looking into the phthalate problem. This is a good example of useful cooperation between the manufacturers, regulatory agencies, and NTP scientists.

In addition to the executive committee, the NTP is guided by its board of scientific counselors which meets several times a year in open session to evaluate the program's scientific quality, review critical programs, recommend needed changes and identify future needs. The board is composed of eight non-Government scientists and is chaired by Dr. Norton Nelson of New York University, a distinguished scientist in environmental toxicology.

In my statement I discuss improvements in the NTP chemical nomination and selection process. In the interest of time, I will move forward.

I said I would discuss the shortcomings of NTP. I shall, but I shall be brief and not necessarily because there are only few shortcomings.

We would like to be moving faster on developing the information needed by the regulators to estimate the risk from various chemicals.

We are looking at the problem of conducting multiple dose rodent bioassays.

Clearly the regulatory process would be simplified if we could tell from animal studies more about the risk from carcinogens and other toxic agents to which we are all exposed.

We know that some are more hazardous than others.

However, the issues involved in generating human risk estimates from laboratory animal data are complex, and for this reason the development of the methodology for making these predictions is coming more slowly than any of us would like.

One promising area of research involves the development of methods to detect early DNA damage.

Conventional wisdom tells us that most cancers begin with the interaction between a chemical and the DNA of a cell. DNA is the material that tells each new generation its structure, form, and function.

This altered DNA then is the first step in the process that leads to cancer, weeks to months later in rodents, years to decades later in human beings.

If we can develop evidence that DNA has been altered—either by chemical analysis of DNA or by the occurrence of somatic mutation or damage to the chromosome apparatus—we can establish a carcinogenic risk soon after chemical exposure.

In laboratory animals this may give us a useful rapid test. We think these methods can be applied to simple blood samples drawn from a human population. If this works, we can detect risks in humans very early indeed.

I also wish we knew more about the effects of mixtures or combinations of chemicals. We all aren't laboratory animals fed perfect diets and exposed to a single test chemical at a time.

Rather, in our everyday lives, we are exposed to dozens, or perhaps hundreds, of potentially toxic agents at very low doses. Some of these are in our air; some in our water; and some in our food.

Very little is known about the effects of such combinations. We simply don't know as much about this as we would like.

Mr. Chairman, NTP serves a number of vital purposes. It tests chemicals and classes of chemicals to see if they present public health problems. It is developing short-term methods needed to better cope with the vast universe of untested and unknown chemicals in which we live.

In this effort, NTP is getting regulators and researchers to talk to each other, to share their knowledge and discuss their mutual problems in a way never attempted before.

The data and the methods NTP develops are needed to protect the public health. NTP is not a regulatory program. It is a scientific research program that works in harmony with regulatory needs.

I am gratified as to the progress in the development of close interaction between NTP and industry toxicologists.

In the past often the mere development of data showing a hazard has resulted in voluntary action by industry to protect the public health before the cumbersome regulatory process acted, or had to.

This happened in the case of bis(chloromethyl)ether, PCB's and DBCP.

Once the hazard became clear, industry acted. NTP exists to develop the information and the tools that both other agencies of government and industry need so that we can all live together safely in the same world.

I would be delighted to answer any questions.

Thank you.

[The prepared statement of Dr. Rall follows:]

### STATEMENT OF DAVID P. RALL, M.D., PH. D.

Mr. Chairman and members of the Committee, I am Dr. David Rall, Director of the National Institute of Environmental Health Sciences (NIEHS) and the National Toxicology Program (NTP). In the latter role I report to Dr. Brandt. I am pleased to appear before this Committee today to discuss the National Toxicology Program and its progress over the last two and a half years.

Dr. Brandt has described how NTP came into being and where it is going over the next several years.

I'd like to elaborate on what I think are the NTP's significant accomplishments in its first two and a half years and touch on a few of our shortcomings.

First, we have made significant strides in improvement in the long-term animal bioassay. Formerly, this only gave us a "yes" or "no" answer as to whether a chemical caused cancer in animals. We are developing an integrated approach to toxicological testing using an expanded experimental design. The ultimate goal is to measure the full toxicological potential for each chemical studied.

Bioassays have been expanded to include studies of genetic damage, reproductive effects, and damage to kidney, liver, lungs, and the immune and nervous systems. We also do hematology and urinalysis. About 70 percent of the 40 chemical starts in fiscal year 1980 included such special studies. All chemical starts developed in fiscal year 1981 have an expanded design.

A major improvement is that by 1982 we expect to study the distribution, metabolism and excretion of most of the chemicals we are testing. This is important because most chemicals are metabolized or chemically altered in the body to different and frequently more toxic compounds. Further, it aids in the selection of proper dosage and dose regimen.

The design of each bioassay is supervised by key scientific staff of the NTP. The protocol for each bioassay is individually tailored to gain as much useful information on the effects of the chemical as possible. An NTP committee monitors these studies as they progress and reviews and approves the draft report before it goes to our Board of Scientific Counselors' Peer Review Committee for independent scientific scrutiny.

We want the final result of our testing effort not to be a mere statement that a chemical does or does not cause cancer in animals, but a more complete toxicological profile, one which evaluates previously published studies found in our literature search, one which looks at a wide variety of possible toxic effects and one which can be published in the literature so that the scientific community can have access to this information without having to write the Government for a copy of the report.

While this process goes on, we make the results of pre-chronic tests and status reports available to interested investigators, regulatory agencies, etc., so that others who are looking at the same problem can have access to what we are doing without waiting the full three or four years this process usually takes.

In addition to making significant improvements in the long-term bioassay, NTP is developing new tests and protocols that are needed to meet future toxicological testing requirements, particularly in fertility and neuro-behavior. We are developing new short-term in vitro tests to allow more rapid screening of chemicals or mixtures of chemicals for cancer potential—short of the two-year rodent bioassay—and to test for other toxic effects.

In the development of new short-term test systems, we want to assure that the test is adequately sensitive, minimizes positives and false negatives, can be standardized and produces results that are reproducible from laboratory to laboratory, and finally yields results that are meaningful in terms of human health.

NTP has supported an international effort to evaluate short-term tests for carcinogenicity which looked at a variety of tests using a number of labs in several countries. The results illustrated the problems we face: several classes of chemicals that are carcinogenic are apparently not mutagenic; certain tests produced differing results, depending upon the chemical under test; and some short-term tests appeared to be more accurate predictors of carcinogenicity than others. These data are still being analyzed.

Another NTP effort is the chemical mutagenesis testing program, which has two primary goals: to determine how different types of chemicals affect genetic structure and to correlate mutagenicity results with those for carcinogenicity. We routinely evaluate the mutagenicity potential for all chemical being bioassayed. So far almost 900 chemicals have been or are being examined for mutagenicity.

In other stages of development are programs to develop short-term tests for neurobehavioral toxicity, impaired kidney and pulmonary function, and a teratogenesis pre-screen. We expect to validate a fertility and reproduction assessment assay during the coming two years. We have also begun an interlaboratory study of an immunotoxicity test battery and have let two contracts for a short-term lung adenoma test for carcinogenicity that if validated should produce results in six months,

In carrying out this task, the NTP is guided by its Executive Committee, composed of the heads of the major regulatory and research agencies. The Committee advises me on research and testing needs; helps nominate, evaluate, and select chemicals for testing and sets priorities for those selected; and oversees program planning and balance of NTP testing and test methods development programs.

I'd like to discuss two recent research initiatives with which the Executive Committee has been involved. The first is an assessment of the toxicology of derivatives of benzidine, a human carcinogen. As the use of benzidine itself as a commercially important dye has decreased, attention has focused on the substitution of chemical derivatives of benzidine. It now appears that these derivatives metabolized; that is, chemically altered by the body so that free benzidine is excreted in the urine. Many of these benzidine derivatives are in use today and only a few of them have been tested for carginogenicity. It is important to determine which of these compounds are converted in the human body to free benzidine, which are mutagenic in short-term tests and which should be tested for carcinogenicity in long-term studies. All of the NTP agencies are collaborating in this effort.

Another research initiative involves the phthalates. These interesting compounds—about 1.3 billion pounds a year are marketed in the U.S.—are used primarily to soften plastics. Phthalates were discovered to be rather ubiquitous in the environment about 1970. Of particular concern is the fact that phthalates were found in hospital blood bags and in the blood used for transfusions. Since this class of compounds had not been tested for long-term toxicity, NTP in the mid-1970s initiated a phthalates testing program. NTP testing now has shown that certain of the phthalates are carcinogenic and certain may have other important toxicity effects.

NTP, the IRLG, and the Chemical Manufacturers Association organized an international symposium in early June dealing with the problems of phthalates toxicity. NTP will serve as the international clearinghouse for the development of research programs looking into the phthalate problem. This is a good example of useful cooperation between the manufacturers, regulatory agencies and NTP scientists.

In addition to the Executive Committee, the NTP is guided by its Board of Scientific Counselors which meets several times a year in open session to evaluate the Program's scientific quality, review critical programs, recommend needed changes and identify future needs. The Board is composed of eight non-government scientists and is chaired by Dr. Norton Nelson of New York University, a distinguished scientist.

I also want to mention improvements in NTP's Chemical Nomination and Selection Process. The decision to test a chemical—and which chemical—is critical to the effective operation of NTP, both with respect to testing using conventional methodologies and to the development and validation of new test methods. Our new chemical selection procedure, which is necessarily quite involved, is an interagency effort involving both the research and regulatory agencies. Nominations for testing are reviewed by NTP's Chemical Evaluation Committee, published for input from the public sector, reviewed by the Board of Scientific counseolrs and coordinated with EPA's Interagency Testing Committee before being acted upon by the Executive Committee. This multi-tiered process is designed to assure that chemical selection is as careful as possible.

I said I would discuss the shortcomings of NTP. I will be brief. We would like to be moving faster on developing the information needed by the regulators to estimate the risk from various chemicals. We are looking at the problem of conducting multiple dose rodent bioassays. Clearly the regulatory process would be simplified if we could tell from animal studies more about the risk from carcinogens and other toxic agents to which we are all exposed. We know that some are more hazardous than others. However, the issues involved in generating human risk estimates from laboratory animal data are complex, and for this reason the development of the methodology for making these predictions is coming more slowly.

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Mr. Chairman, NTP serves a number of vital purposes. It tests chemicals and classes of chemicals to see if they present public health problems. It is developing short-term methods needed to better cope with the vast universe of untested and unknown chemicals in which we live. In this effort, NTP is getting regulators and researchers to talk to each other, to share their knowledge and discuss their mutual problems in a way never attempted before.

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I would be delighted to answer any questions.

Thank you.

Mr. GORE. Thank you very much.

We have a vote on the floor. We are going to go over and come back.

Let me just ask one brief question before we go. It is provoked by your last comment about the excellent relationship with industry and the NTP.

Why, in your opinion, would the industry not wish to participate voluntarily in a proceeding like this to evaluate the NTP and to try to come up with commonsense improvements to it?

Dr. RALL. I really have no idea. I must say I fully expected them to be here tomorrow and give their point of view.

Mr. GORE. Yes. Well, that is too bad.

This is a natural breaking point. We will come back and then have some questions. We ask your indulgence.

We have a vote on the floor. We will be back in about 7 or 8 minutes. [Recess.]

Mr. GORE. The subcommittee will come to order.

Dr. Rall, in previous testimony you said that although the NTP funding arrangements were awkward, you would recommend against moving NCI funds into the NIEHS. Has anything happened in the past year to change your mind on this?

Dr. RALL. Yes, sir, a lot has happened.

Dr. DeVita suggested we ought to reexamine that issue and Dr. Frederickson, Dr. DeVita, and I, reexamined the issue and Dr. Frederickson thought it would be a good idea for administrative purposes to propose that we move the NCI funding for what was formerly the bioassay program to NIEHS.

Mr. GORE. What is the status of that proposed reorganization?

Dr. RALL. It was just recently approved by Secretary Schweiker.

Mr. **Gore**. I want you to fully understand that we are interested in getting your candid views on these matters because what we are trying to do is to make sure this program is working in an optimum way. Is that a good idea, in your judgment?

Dr. **Rall**. It will make my life and Dr. DeVita's life quite a bit easier. I am selfish enough to think that is good. I think in the long run it is very good. I was concerned and I was a little reluctant to propose it because the National Cancer Institute does have a mandate to worry about chemical-caused cancers, and if the perception appears that by giving up the bioassay program, NCI is giving up their concern with chemically caused cancers, then I think that would be too bad.

I think, however, it is clear now that NCI is doing enough in its prevention programs that that is not necessary, and I think that potential disadvantage will probably not occur. I am basically very pleased now.

Mr. **Gore**. Let me welcome to the witness table at this point our last three witnesses, Dr. Vincent DeVita, Director of the National Cancer Institute; Dr. Ronald Hart, Director of the National Center for Toxicological Research; and Dr. J. Donald Millar, Director of the National Institute for Occupational Safety and Health.

We welcome you gentlemen.

Mr. **Gore**. At this point, Dr. DeVita, I was going to wait to go to those other questions, but let me ask you at this point to comment on Dr. Rall's last response.

Do you agree with it?

## STATEMENTS OF DR. VINCENT DE VITA, DIRECTOR, NATIONAL CANCER INSTITUTE; DR. DONALD HART, DIRECTOR, NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH; DR. J. DONALD MILLAR, DIRECTOR, NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH

Dr. **DeVita**. Yes, sir, I do. I think that for purposes of management it is better to have a program of this size requiring this much day-to-day management of the flow of chemicals under the direction of one program director.

For that reason, and also for the reason that the characterization of the toxicology of the chemical was broadened as a result of the NTP and the broadening was the directly within the mission of the NIEHS, I felt it was appropriate to suggest that these resources be transferred.

I was asked by Dr. Frederickson to discuss the actual transfer of resources with the National Cancer Advisory Board. They fully agreed with the suggestion that we transfer these resources.

Mr. **Gore**. Dr. Rall, if it is a good idea to transfer the NCI dedicated money in the interest of tight management, should the NCTR and the NIOSH funds also be transferred to NIEHS for the same reason?

Dr. **Rall**. I don't think so, in all honesty. With NIEHS—NCI as the so-called NIH part of NTP, you have two research agencies whose mandates are to further the knowledge in these areas of human diseases.

It is much more interchangeable, whether we do the research and it comes out under NIH—NTP or whether NCI does the research and it comes out under NCI's imprimatur.

When you go into NIOSH, which has a congressionally mandated job to support occupational safety and health, then that is a rather narrower mission. It makes me nervous to be involved with that; similarly with NCTR, whose mission basically is to support the Food and Drug Administration.

The major missions of these two agencies must be dealt with. I think it could get very confusing if a research agency were in charge directly of significant amounts of the research effort of these two agencies.

I have always felt very strongly, as I have discussed about the organization of environmental health research in the Government—which I have been doing since 1972—Although the research agencies should do the bulk of the research, it is absolutely critical that each regulatory agency have its own adequate and adequately staffed research component so that the head of each regulatory agency has his or her scientists whom he or she can go to for advice, for counsel, and to do critical urgent experiments.

So as I explained, I am comfortable with the NCI transfer. I would be uncomfortable with the others.

Mr. GORE. Doesn't the current arrangement create a little confusion in management?

Dr. RALL. I think it creates a little confusion in management. If we can keep it to only a little confusion, I think we will be doing OK.

Mr. GORE. It is important to have realistic objectives.

Dr. RALL. I think it should be noted that with the consolidation of the NIH—NTP, that now accounts for 85 percent of the fiscal resources of the program.

Mr. GORE. Dr. Hart, let me ask you as Director of the National Center for Toxicological Research, does the current arrangement create confusion in your view?

Dr. HART. Well, hopefully a minimal amount of confusion. I think the NTP has to be viewed as an evolving program. It is a relatively new one. It is a new concept, and anytime you have an evolving program you are going to have some degree of confusion.

In order to overcome this, Dr. Rall and I have established a telephone conversation every 2 weeks. We also are committing ourselves to a meeting every 3 weeks in Washington to discuss programs and policies within the programs. I feel this is a very healthy approach.

If we had agreed on all aspects of all approaches to the program, this would simply mean that the NTP wasn't evolving but rather stagnating. I think it is better in the long run to evolve.

Mr. GORE. When did you set up this regular telephone call, regular visit arrangement?

Dr. HART. I think it was what——

Dr. RALL. Eight months ago.

Mr. GORE. Has it worked out pretty well?

Dr. HART. Very well so far.

Mr. GORE. At least on the surface many of the objectives of your Center for Toxicological Research would seem to overlap the objectives of the NTP.

Could you clarify for us and for the record what the differences are between the research objectives of NCTR, your group, and NTP?

Dr. HART. I think this was already addressed, previously, by the GAO. They did not find any overlap in programs.

The thing is that the field of toxicology is an immense field, an extremely important one.

There is probably more work in this field yet to do than any of us could do in our lifetimes and probably in several other lifetimes.

If you look at, for example, Dr. Rall's testimony, or Dr. Brandt's testimony, he lists the goals of the NTP.

These are not necessarily overlapping with the goals of NCTR, except for our mission to do chemical evaluations.

Our chemical evaluations are not just for cancer end points. They are rather broad brush evaluations or emergency response situations.

For example, a few months ago the head of the Bureau of Biologics requested that we do a rapid toxicological evaluation on Proven. This is a substance I am sure we are all familiar with and have heard about.

We had to do a complete animal assay in a relatively short time frame.

Mr. GORE. What is this substance?

Dr. HART. Proven. It is a substance which is being issued in Florida or presumably has been issued in Florida in the past and is a presumptive treatment for multiple sclerosis and arthritis. It is under investigation by the FDA at this point.

We could not respond—we had to respond in an extremely rapid manner with the facilities we had available.

So the types of research oftentimes we do involve chemical testing, but they are not exclusive only to chemical testing.

Our other three missions are to develop methodologies which allow us to make better risk assessments, develop an understanding of how we can extrapolate animal data to human health effects, and evaluate the basic mechanisms of underlying toxicity. These are not necessarily overlapping with NTP's missions.

Mr. GORE. My time has expired. I will continue on a second round.

Mr. Walker?

Mr. WALKER. Thank you, Mr. Chairman.

Dr. Rall, at the end of your testimony you did mention the cooperation that you have had with industry toxicologists. Is that a relationship now where you do have industry people who will indicate to you in advance that they think there is a chemical that you should undertake research on?

Is that the kind of working relationship that you have or are they cooperative only when you identify a chemical and then you indicate that there are some problems and at that point you find them cooperative?

Dr. RALL. The first sort of example you gave, where they think there is a problem with the chemical and they come to us, puts them in a very difficult position because one of the sections of the Toxic Substance Control Act strongly suggests that they should come to EPA promptly if they think there is a problem with one of the chemicals they are involved in.

In fact, that is what they typically do.

They come to us for advice and consultation, methodology, sometimes interpretation. I think the experience with the phthalates which are a very important group of chemicals is indicative of this. We can lean back on these chairs. The plastic is soft and pliable because there is phthalic acid ester in there making the plastic soft.

If, in fact, these turn out to have serious toxicological effects, we have a lot of problems trying to find substitutes.

Working together with the representatives of the Chemical Manufacturers Association, with the various specific companies, we put together a program, did a nice job of reviewing the problem, laying out where we ought to go in terms of research, and creating a mechanism for following up on that.

We involve industry in chemical nominations. We have a member of the Chemical Industries Institute of Toxicology on our Peer Review Group. We have a person from Dow on that group too.

We do work very closely.

Mr. WALKER. Do I understand from your testimony you said almost 900 chemicals have been or are being examined for mutagenicity and that compares with about 7,000 that have been tested for carcinogenicity; is that approximately correct?

That is out of the 55,000 or so known chemicals, is that true?

Dr. RALL. The 900 we have tested in our own program, which is a very carefully controlled testing program where we run chemicals blindly. We have known positives and known negatives.

The 7,000 chemicals that have been tested for carcinogenesis is a worldwide estimate.

Many of those tests are not of good quality, often because they were done ten or twenty years ago when the state of the art was such that that was it.

I would estimate that there probably have not been more than 2,000 good tests.

We have these sorts of question of how many chemicals are carcinogenic, how many chemicals are toxic, how many have been tested, how many haven't been tested, that I think we desperately need answers to, and we just do not have them.

About a year and a half ago we began negotiations with the National Academy of Sciences to develop what we call a testing needs study. We now have a contract with them and almost the first year is out and they are moving very well.

The purpose of this study is to list all the chemicals that are in production or that we are likely to be exposed to, whether they be food additives or drugs or whatever.

Then take a random sample of those chemicals, get it down to a manageable number, and have knowledgeable groups of scientists look at those chemicals and ask a number of questions.

Are we likely to be exposed to them or is this some laboratory curiosity that six people in the country are exposed to?

Has there been adequate toxicology data done, and so forth? They can then come back and by estimate using statistical methods the nature of the testing problem.

We hope to have that resolved in about 18 months. I think that will be very useful.

Mr. WALKER. Do you have any idea of what the costs of conducting such a study might be if you get it down to manageable levels?

Dr. RALL. The testing needs study?

Mr. **Walker**. Well, I am talking about once you get past the study and move into examining a reasonable number of chemicals on a random basis. Do we have any idea?

Dr. **Rall**. We are not going to do the tests. We are going to go into the literature and see if they have been tested.

Mr. **Walker**. This will give you some idea as to the percentage of chemicals then that have had some kinds of tests along the way?

Dr. **Rall**. Right.

Mr. **Walker**. I would like—what concerns me in this whole area, as it concerns me generally in the area of regulation and seeing sometimes the dearth of research that has been done, is that we are too often developing both here in the Congress and within the regulatory agencies a large body of law and regulation which has very little scientific basis.

I would appreciate from whoever wants to respond to it some characterization of where we are right now with regard to toxic chemicals.

Are we able to adequately regulate at the present time, particularly in those areas beyond which we have test data?

Dr. **Rall**. It is a very serious problem. You have heard us emphasize the need for short-term rapid tests. I think the Toxic Substance Control Act was passed by Congress and signed by President Ford, believing that we had available, off the shelf, short-term tests that would let chemical manufacturers quickly run through their compounds and say this looks pretty good, this looks bad, this looks bad, these three look good. We are not quite there yet. We are getting there.

I would think that with the efforts we are making supporting academic scientists—NCI's funding a lot of this research—in a couple of years we will have a good sort of first generation battery of tests.

Hopefully then EPA and toxic substance control folks can begin to encourage industry to use those before chemicals are marketed.

Then we can reserve our expensive, our 2-year, studies, for only those critical compounds that we really need in our technologically oriented society, but which may be carcinogenic.

Then we test those and do risk assessment. You have heard Dr. Omenn and myself describe how hard risk assessment is.

Mr. **Walker**. Do you find anything in the law or the regulations right now which is a frustration rather than a help in terms of getting about the job of getting adequate research and then putting good regulation into effect based upon that research?

Dr. **Rall**. I can answer for the research part of that. My answer is no.

I would be delighted to refer to the more regulatory-oriented people which is Dr. Hart, I guess.

Dr. **Hart**. Well, I would say we are supposed to be primarily a research operation, so I would defer to Dr. Brandt.

[Laughter.]

Dr. **Brandt**. I think——

Mr. **Gore**. We have some regulators coming in tomorrow.

Dr. **Brandt**. That is just what I was going to say.

Mr. **Walker**. Generally, you do not think there is anything in the law and regulation right now which is frustrating the research efforts?

Dr. **Rall**. No.

Mr. **Walker**. Thank you, Mr. Chairman.

Mr. **Gore**. Thank you.

Mr. Shamansky.

Mr. **Shamansky**. Thank you.

Has the transfer of the National Cancer Institute, Dr. DeVita, bioassay program to the NTP had an effect on the ability of the National Cancer Institute to retain or recruit high quality staff?

Dr. **DeVita**. No, sir. I don't believe it has. Actually, for the past 18 months the program has been managed effectively by Dr. Rall and his staff.

The Cancer Institute has not been recruiting staff for this particular program.

The NTP has. I think that there have been some difficulties involved in the uncertainties of where the program was housed that may have inhibited their recruiting efforts. Perhaps they would like to comment on that.

But from our point of view, no.

Mr. **Shamansky**. For you and Drs. Hart and Millar, in what areas of toxic substances research do you feel your agency possesses a unique expertise and thereby should remain a part of your agency's research activity?

Dr. **DeVita**. We look at our mission in the area of cancer etiology as the discovery of the cause of cancer.

In the process of doing this, we may uncover methods of evaluating the effects of chemicals on causing cancer.

For example, much of the new DNA recombinant technology offers us a way of looking at damage to DNA, namely in a piece of mammalian DNA stuck in a bacteria. However, the people who discovered that and developed it, were not looking at tests for evaluating the carcinogenicity of chemicals.

I do believe that our mission to continue to look for the etiology of cancer will continue to serve the NTP as long as we are members of the executive committee and on its Board of Scientific Counsellors so the information flow will continue.

We still retain that relationship with the NTP.

Mr. **Shamansky**. Everything has seemed so harmonious this afternoon. I am getting a little worried.

I came to this job with these marvelous stories about everyone fighting for his own turn. You fellows are all such gentlemen about the whole thing. It leads to two possibilities, it seems to me: Either that everything is going along marvelously, or you are doing a heck of a con job on us here, which I do not think you are doing.

Dr. **DeVita**. May I comment on that?

Mr. **Shamansky**. Sure.

[Laughter.]

Dr. **DeVita**. I would not want to leave you with the impression that we haven't debated all the ins and outs of the organization. We have.

Mr. **Shamansky**. This is what I am trying to find out. If there is something here we can help you with, we want to do it.

Can you tell us what your basic concerns are in getting along with each other?

Dr. DeVita. As far as management was concerned, it was clear to me when I became NCI Director 18 months ago, that it was not going to be efficient for Dr. Rall and I to try to make the same decision twice.

We decided that he would operate the NCI resources. Having decided that, it didn't seem too big a step to make the necessary transfer.

The big debate that took place in our community was whether removing the testing program from the science base in the Cancer Institute would be detrimental to the testing program.

In other words, you would lose this contact. Our retort to that was, if you move the testing program from one science base, the cancer science base, you would merely be moving it into another albeit smaller science base at NIEHS.

We thought the move imperfect, but you can't keep both of them together. No matter what you do, you lose a little something. It took a lot of debate to get to that point.

We have had heated arguments about whether you should or should not do this.

I think at the moment we are very comfortable with the present relationship. I think I am going to have a job explaining to the Congress, as Dr. Rall mentioned, and to many people, that we have not diminished our interests in the prevention side even though our budget line in the prevention thrust will have dropped by some $46 million or more.

We have not changed our emphasis. That particular piece of money has just changed to a different place.

Other than that, I don't see a problem.

Mr. Shamansky. Would any of you other gentlemen like to comment on that?

Dr. Hart. If the question still relates to what type of research activities—which was the initial question—I think NCTE has some very unique capabilities, both facilities-wise and research-wise.

Included among these are No. 1, teratology. That is the study of the effects of chemical agents on developing fetuses and embryos. Both morphologically, as well as physiologically and behaviorally.

For example, certain chemical agents which will cross the placenta during birth have significant impact not only on the morphology of a developing embryo, but also on the behavior in the future of the exposed individual.

The second area which we have, I think, some degree of expertise in, is the development of computer systems for management of toxicological programs which are also being developed in coordination with NTP.

A third area where we feel we have fairly strong emphasis is in our chemical analysis area, especially for metabolism, mass spectrophotometry, which is a way of analyzing very small amounts of a chemical compound.

Our fourth area of expertise lies in the development of in vivo, hopefully predictive biological markers for genotoxicity, or, in other words, the identification of chemical agents which will damage the DNA which we have heard so much about today.

That happens to be my own field of interest and expertise.

Mr. **Shamansky**. Are you satisfied that you have, if not the most rational system, a workable allocation of duties and resources to handle the challenges presented to the research establishment of the Federal Government?

Dr. **Hart**. I think at this point that it is still an evolving process.

Mr. **Shamansky**. I understand that.

Dr. **Hart**. I think we are evolving, especially under Dr. Brandt, to a more and more reasonable evaluation of resources and activities. I have been very impressed by the decisions and the way that things have been able to work in this very complex field since my joining the Government all of something like 10 months ago.

So I am——

Mr. **Shamansky**. I have only been here 6 months, so I understand the feeling.

Dr. **Hart**. Since we both come from the same district in Columbus, Ohio, that is quite reasonable.

Mr. **Shamansky**. Dr. Millar?

Dr. **Millar**. I wanted to respond to your initial question about unique aspects of the various programs. The NIOSH program is unique in that it responds to field investigations of acute problems rather than being principally oriented around basic research.

We are quite happy with this arrangement within NTP. Our working laboratory people have found it quite useful.

Our toxicologists have appreciated the exposure to other toxicologists in the working committees, so we are quite happy with it.

We think it is a good example of a matrix organization that really works.

Mr. **Shamansky**. Is the transfer of the NIOSH operation to Atlanta a big problem for you?

Dr. **Millar**. It should have virtually no effect on the national toxicology aspect which is located in Cincinnati.

Mr. **Shamansky**. That is a good place for it. Better than Atlanta.

Mr. Chairman, I think everything is perfect based on the testimony.

Mr. **Gore**. All right.

Dr. Rall, do you support the findings of the Federal Formaldehyde Panel headed by Dr. Richard Griesemer which indicated that formaldehyde is an animal carcinogen and "should be considered as posing a cancer risk to humans?"

Dr. **Rall**. Yes, I certainly do.

Mr. **Gore**. Dr. DeVita, do you support that finding?

Dr. **DeVita**. Yes. I think by our definition it is a potential human carcinogen.

Mr. **Gore**. Dr. Hart, do you support it?

Dr. **Hart**. I agree that it is a potential human carcinogen.

Mr. **Gore**. Dr. Millar, do you support that?

Dr. **Millar**. I agree it is a potential human carcinogen.

Mr. **Gore**. It seems that the leading scientists agree in the Federal community with that result.

Dr. Rall, are you familiar with the procedures at the IARC, this international group?

Dr. **Rall**. Yes. In general, not in detail.

Mr. **Gore**. Is it fair to say that the amount of evidence on formaldehyde which they considered was more limited than the amount of evidence available to those toxicologists and scientists in the Federal Government here in the United States familiar with the matter?

Dr. **Rall**. I think so, but we may be dealing with a question of at what point in time. I don't know—and I didn't go back and find out—when the working group met to decide on the potential carcinogenicity of formaldehyde.

The major evidence that formaldehyde is carcinogenic is from this study coming out of the CIIT, the Chemical Industries Institute of Toxicology, which has been evolving and which, in fact, still is not completed.

About a year ago were the first indications of tumors at the single high dose in rats. We now know that the next lower dose had some of these unusual tumors and the high dose of the mice had it. I don't think there is any question that if the IARC working group looked at all the data now that they would say there is sufficient evidence. Where this happened, and who knew what when, I just do not know.

Mr. **Gore**. Well, I do know a little bit about that. I think the record will reflect that the IARC chooses not to include in the evidence considered the work of the Federal Formaldehyde Panel.

Would you agree that the findings of the Federal Formaldehyde Panel constitute at this point the most up-to-date and thorough statement on this matter?

Dr. **Rall**. That is right, but it is perfectly right for the working group not to include Dr. Griesemer's report because that was not original research. That was a review of what was available and their interpretation of the review.

The working groups typically do not deal with such subjective views.

Mr. **Gore**. Of course, I think the record will also reflect that there is some confusion about the exact definitions used by the IARC and it is open to the interpretation that this sort of study can be used, but the point that I wanted to establish is that all of you gentlemen with expertise in this area agree with the findings of the Federal Formaldehyde Panel that formaldehyde is an animal carcinogen and "should be considered as posing a cancer risk to humans?"

So it is not irrational for some other scientist in the Federal research and scientific community to reach that conclusion?

Dr. **Rall**. That is right.

Mr. **Gore**. I have several more questions.

I will ask the indulgence of the panel because we have the defense bill on the floor and we will recess just briefly.

We will reconvene in about 7 to 8 minutes.

[Recess.]

Mr. **Gore**. The subcommittee will come back to order.

We are going to try to finish up here before too long.

Dr. Rall, the Department of Health and Human Services and the National Toxicology program are to be commended, in my opinion, for their efforts in coordinating toxicology research within a sizable part of the Federal Government and for recognizing a problem of growing concern. However, such a program as the NTP also raises the question of whether regulatory agencies can shift their research needs to the NTP and thus eliminate much, if not all of their needs for in-house research programs.

How do you feel about this? Do you believe that the NTP can effectively substitute for the toxic research currently being performed by and within the regulatory agencies?

Dr. **Rall**. Mr. Chairman, I do not think so.

As I said before, I think each regulatory agency, each regulator, if you will, must have at his or her fingertips a competent cadre of scientists, deeply knowledgeable in the area to be regulated.

I think that is a necessity.

Above and beyond that, I think the research agencies typically can do better research—and don't jump on me until I finish what I am saying.

When you are in a regulatory agency you must respond to immediate, sometimes terrible situations.

If you were in FDA and there is a shipment of green beans contaminated with botulism toxin, a long-term research project is the last thing you want to worry about.

You want to find that green bean shipment and get it off the shelves and warn the people. It is very hard for a regulatory agency to maintain the long-term research that research agencies can and do well.

I think there is a neatly balanced and complementary way: regulatory agencies have significant amounts of research at their beck and call, research agencies do primarily the longer term research, but are also able to help with the short-term research when needed.

Have I given an adequate explanation of my views?

Mr. **Gore**. Well, is that the only exception you would carve out, emergency response, and similar things? Do you think——

Dr. **Rall**. I think both agencies, both types of agencies help each other when the situation arises, but the premise I really come back to is that the regulatory agency needs a certain size cadre of their own scientists, that above and beyond that, for long-term research, research agencies do better.

You need a mechanism to keep them relevant. That is why we designed the executive committee which has the four health regulatory agencies sitting advising me, sometimes very strongly, as to what they think we ought to do and where they think we ought to go.

Mr. **Gore**. Do you think that the CPSC chronic toxics program should be transferred to the NTP?

Dr. **Rall**. It can't be, because that is a regulatory program, with just a little bit of contract supported research.

Mr. **Gore**. OMB thinks that NTP can substitute for the chronic toxics program at CPSC. Do you disagree with OMB? That is always a fun question to ask.

Dr. **Rall**. Never let the record show that I would disagree with OMB. I am sure Dr. Omenn is sitting here.

I have heard——

Mr. **Gore**. They didn't do it when he was there.

Dr. **Rall**. I know. He and I had other disagreements.

That is what I have heard. I have not seen it. I didn't hear the testimony. I just don't know how I can comment on that.

Mr. GORE. Well, your testimony, it seems to me, can be interpreted by a reasonable person to mean that you disagree with a proposal to eliminate the CPSC chronic toxics program on the assumption that NTP's work can substitute for it?

Dr. RALL. That is correct.

Mr. GORE. OK. That is close enough.

What do you believe NTP's role should be in the determination of risks of toxic substances? Should NTP or could NTP be the body to effectively oversee questions of scientific judgment?

Dr. RALL. Well, risk assessment is a multistage process, as Dr. Omenn discussed. There is the first stage, the identification of the possibility of risk.

The second stage is the development in a laboratory and at the epidemiological level of some estimations of the risk.

Then you move into the third stage, which is exposure, modes of exposure, numbers of people exposed, and so forth.

Then you get into some sort of attempt to balance off risk and benefit.

I think NTP can do quite well at the first two; risk identification and, at least in terms of the laboratory animals study, some greater effort to in some way quantitate the risk.

I think that is where on a routine basis we should stop. We do have very good biostatistics; we have some imaginative scientists. We would like to, on an exploratory basis, move a little further in that process, but I think it still should be the regulatory agency's job to make that final determination of their best estimate of the risk to man and the beginning of the balancing act which is necessary.

Each regulatory law has fine nuances of differences, and I think if NTP tried to get involved in that we should be in trouble before we knew what happened.

I think we should take the first part of it, help the agencies with that; the second part should be the regulatory agency's responsibility.

Mr. GORE. So you would separate the scientific judgment from the regulatory judgment and you would be willing to take the scientific judgment?

Dr. RALL. That is right.

Mr. GORE. How would most of the regulators react to that kind of breakdown?

Dr. RALL. If it is a popular decision, with sadness. If it is an unpopular decision, with joy.

Mr. GORE. I think I got that. [Laughter.]

I think they did too.

We will ask them tomorrow at any rate.

Background figures on the NTP's operations also indicate that about 59 percent of NTP's resources devoted toward chemical testing in fiscal year 1981 will be spent on carcinogenesis testing. While cancer is indeed one of the most dreaded diseases with which we have to cope today, the chemicals evaluated by the NTP are also suspected of producing a number of other adverse human health effects.

The Kepone incident at Hopewell, Va., is one that comes easily to mind.

What is the NTP doing in terms of testing chemical substances for other toxicologic end points such as neurobehavioral effects? Do you expect the NTP to continue spending 59 percent of its testing resources on cancer testing or will this percentage change over time?

Dr. RALL. This percentage is changing over time.

It is steadily decreasing. It is that high because we are faced with about 200 compounds which were put on test prior to 1979 and we have to finish those tests.

The ones we are designing now are different. They have more end points under study than just carcinogenesis. By the time the full cycle of testing has gone through something like 1984, or 1985, we will probably be spending more like 33 percent on carcinogenesis.

Dr. Moore, is that a reasonable estimate?

Dr. MOORE. Yes.

Mr. GORE. Do you think that is about optimum?

Dr. RALL. About.

Mr. GORE. Anybody on the panel disagree with that?

Dr. DEVITA. No.

Dr. HART. No.

Mr. GORE. Dr. DeVita?

Dr. DEVITA. I am not sure that we can be precise about an optimum figure, but we are satisfied in our interactions with the NTP that our testing needs capacity is being met.

It is interesting, when we were running the bioassay program for cancer only, a lot of the testing capacity was being wasted in the sense that there were no end points other then cancer being measured.

While a lot of that money is going for carcinogenesis, a lot of other end points are being measured in the process now. So the 59 percent figure is probably an overestimate.

Mr. GORE. So we have allocated too much money to the carcinogenesis assessment?

Dr. DEVITA. No. I meant that when you tested a certain number of chemicals and only measured for one end point—that is, the ability to cause cancer—you wasted the ability to judge all the other effects of those chemicals on those particular animals. That broader testing was not the mission of the Cancer Institute.

But the NTP is doing that, so, results of other tests are coming things out of those long-term tests earmarked for carcinogenesis.

Mr. GORE. It is obviously cost effective to do several screens simultaneously?

Dr. RALL. May I comment briefly on that?

Your question really is very hard to answer. I think one of the basic premises of NTP was that if it cost $500,000 to test a compound for carcinogenicity, for another $100,000 you could test it for a whole variety of other end points and learn an enormous amount about that compound.

That extra $100,000 bought you an awful lot.

When you then do the complete test, which costs $600,000, how much do you allocate to carcinogenicity? I don't quite know how.

Mr. **Gore**. Do you think we have allocated too much within that amount?

Dr. **Rall**. No, no. How do you allocate—a test costs $600,000 to look for carcinogenicity, and so forth and so on. It is bookkeeping that I can't quite do.

Mr. **Gore**. I have had an opportunity to get into the theory of joint allocation of common costs with accountants. It is a hopeless exercise because there are no set and established rules.

I understand the nature of your problem.

Dr. **DeVita**. It seems to me that there is no question that we are getting more out of the dollar than we put into those long-term tests at the present time by virtue of doing all these multiple tests. That is again one of the basic tenets behind the creation of the NTP.

Mr. **Gore**. As I understand it, the NTP spends about 88 percent of its resources extramurally, primarily contracts, one of which is the familiar Tracor Jitco prime contract previously under the NCI bioassay program.

Is NTP doing a better job of monitoring the Tracor Jitco contract and subcontracts than has been done previously and do you believe a subsequent GAO review would likely give it a clean bill of health so to speak?

Dr. **Rall**. We have been very, very concerned about the problems with Tracor Jitco and we shared NCI's concerns and completely agreed with the NCI decision in May of 1979 to phase out the prime contractor.

All new starts for chemical toxicity testing are done by our own scientists monitoring our own contracts. However, the Tracor Jitco organization is still following compounds begun prior to May 1979 and that will continue until May 1983.

In the interim, we have greatly expanded our surveillance program with Tracor Jitco. The project officer visits Tracor Jitco once a week and they are on the telephone much more often than that.

Laboratory monitors, who are scientists within NTP responsible for the performance of a specific laboratory that Tracor Jitco has contracted with, visit each contractor twice yearly to review the work.

Now, in all honesty, travel limitations have cut that from four times a year, which is what we would like, to twice a year, but twice a year is pretty good.

The so-called disciplines, pharmacology, toxicology, pathology, and so forth, meet with the Tracor Jitco scientists at regular intervals, presumably around 1 month intervals.

So in essence we are doing a much more careful job of monitoring. We think Tracor Jitco is performing better. We review every 4 months their performance on the award fee determination. If GAO would give us a perfect mark, I think it would be wonderful. I think they would give us at least 95 percent.

Mr. **Gore**. OK. You mentioned the cut in travel funds and the staff, and the problems within that poses for monitoring the contracts.

Are the so-called nontravel monitoring devices, such as premeasured double blind samples, an effective substitute for a site visit?

Dr. Rall. That is a very difficult question. We use that extensively in our mutagenesis program where each mutagenesis test is relatively inexpensive.

I am not sure I would have the courage to try to defend setting up an $800,000 double blind study. Ideally it would make sense. It would be a very good way to do it, but that is an awful lot of money just to check something out.

Mr. Gore. You think four site visits a year is probably a much better way to do it?

Dr. Rall. It really is. We get their reports of their work. Our folks in the NTP really understand toxicological studies and they are able to tell whether these studies are being well conducted or whether they are being sloppily conducted.

Mr. Gore. It appears that NTP has made significant strides in terms of opening up the scientific purview process for NTP output and in getting thoroughly unbiased purview.

However, some critics of the process allege that in a few cases insufficient review time has been accorded the peer reviewers prior to the peer panel meeting. A case in point is the chemical chrysotile asbestos reviewed at the June 23, 1981, peer panel meeting.

How much time was accorded to the reviewers in this case?

Dr. Rall. Less than 7 days.

We clearly pushed too hard and we didn't make it. We are sorry.

Mr. Gore. Would you agree——

Dr. Rall. On the other hand, that was the day of the potential air traffic controllers' strike.

Mr. Gore. I remember that day very well.

Would you agree that the perception that such allegations may be true can do almost as much damage to the NTP's reputation as the facts themselves?

Dr. Rall. I really do not think so. Those reviewers are a very independent group of people. They have the prerogative to say, "I will not review it until the next meeting."

In fact, occasionally they say, "Hey, we have some problems with this, not time problems, but something else. Let's bring it back up at the next meeting."

So I think that panel, as constituted, has a proper degree of independence to handle itself in those situations.

Mr. Gore. What impact on the development of the NTP testing protocol do regulatory agencies currently make? Are there situations where the specific needs of the regulatory agencies conflict with the NTP's desire for broader test results, and, if so, how are these situations resolved?

Dr. Rall. I am not aware of any.

Dr. Moore, are you?

Dr. Moore. No.

Dr. Rall. We have altered our protocols for various specific problems. There was some discussion of the two dose protocol. I just remembered that the chrysotile protocol you described was a one dose because this was to answer the question, "Is orally ingested asbestos carcinogenic?" We all know that inhaled, asbestos is carcinogenic.

Orally, nobody knows. It is an important question because of the reserve mining situation in Duluth, the fact that we have asbestos pipes underground where most of our water is carried, and finally in the natural water supply, the water trickles through geologic formations that put chrysotile asbestos into the water.

We used only one dose because we really wanted a yes or no. By having a large number of animals at one dose, that gave us the most powerful sort of response. That is what the regulatory agencies wanted in this instance.

Mr. GORE. Did you get a yes or no?

Dr. RALL. Initially no, but there are still two more studies to go.

Mr. GORE. I have a particular interest in that because I have had specific requests from several hundred constituents about that very question.

Dr. RALL. Are you on our distribution list?

Mr. GORE. I would like to be. I would like to be if somebody here with you can make a note of that.

You mentioned in your testimony today that many complex problems remain to be resolved in the area of quantitative risk assessment.

In your opinion, how far have we progressed in this area? What are the obstacles that remain to be overcome before quantitative risk assessment is a usable regulatory tool? What is NTP doing to advance the state of the art in this area?

Dr. RALL. Well, there are many, many obstacles. People are different. One person from another. You look around this room. We know that.

So, what might be a very serious dose for one person might have very little effect for another.

Generally we have to use laboratory animal studies as the basis for extrapolating and projecting human risk. We know that mice and rats aren't people and in fact they look very different to us and appear very different. So there are enormous differences. Yet we are trying to develop methodology to take information gained from mice and rats and so forth and project to a very complex and diverse human population.

On the other hand, mice and rats biochemically, psychiologically, pharmacologically possess many, many more similarities to the humans than differences.

What we have to do is have enough examples of actions of chemicals, their effects, their metabolism by the mouse, the rat, or the people, to get a much better perspective of how close we are in rats and mice to man.

We are doing that. We are accumulating that information. Our drug metabolism studies are proceeding.

We then need mathematical models to try to estimate what is happening in terms of the dose response curve. Does it seem to be one hit, multihit, this sort of technical business?

We are getting that information slowly.

Finally, I think we are simply going to have to convince people that a certain amount of faith is necessary; that if we try to extrapolate from laboratory animals to man, we will not succeed perfectly every time. We will perhaps succeed perfectly 95 percent of the time. We will have occasional failures. I think we need the scientists, the legislators, the executive branch, and the public, to understand that because we are going to fail occasionally if we use risk estimation. And yet I think ultimately we have to.

We are perfectly willing to let the weatherman on the "Today Show" tell us whether we should wear our rubber boots or carry an umbrella, but we know he fails occasionally.

I think risk assessment, once we overcome these problems, will be much the same. It will work most of the time, but not always; and we will have to learn to live with the failures and develop methods, checks, and balances to catch our failures as soon as possible.

Mr. GORE. Do you think that quantitative risk assessment has advanced to the point where it ought to be included in the development of regulations or is it still in a relatively primitive stage?

Dr. RALL. I think it ought to be done as an exercise, as a learning exercise, at the moment.

Again, I do not want to comment on that too much because I am not that familiar with the specific regulations that the various agencies have to work under.

Mr. GORE. Just as a matter of general principles, do you think that risk assessment is reliable enough and advanced enough to be incorporated on a mandatory basis as part of the formal regulatory process?

Dr. RALL. I don't think it is quite yet.

Mr. GORE. No?

Dr. RALL. Not quite.

Mr. GORE. That would lead me to conclude that a rigid risk benefit analysis incorporating quantitative risk assessment as a prerequisite for any further regulatory action would be unwise. Would you agree with that?

Dr. RALL. I think if you view it as a formal, precise exercise, it would be unwise.

Mr. GORE. That is what we are doing.

Dr. RALL. If you would use it to suggest priorities, to suggest which areas you want to worry about first, then I think it probably would make sense.

Again, I would say that I would like to see this done in a number of instances as a learning process, so we really see where we go if we try it, because I am not sure we have thought out all the byways and the problems that we could run into.

Mr. GORE. Well, the line of questioning is aimed, of course, at the OMB requirement for all regulatory agencies. I think that this inquiry points up the need for better methods. The OMB approach might be quite feasible and desirable if it could be done. There would be other questions that one would want to address, but that is the first one surely.

It leads me to this point: You are spending 83 percent of your resources on testing, but only 13 percent toward the development of better methods, with the remaining 6 percent I guess going to overhead.

Is that an optimum breakdown?

Dr. RALL. No.

Mr. GORE. Shouldn't we be devoting a relatively greater amount toward improving the development of better methods?

Dr. **Rall**. I agree completely. We are moving in that direction as fast as we can and still get the maximum information out of the compounds that are already committed on test. I think everybody would agree if we could do good risk assessment, and have confidence in it, nothing would be better. It would let us really maximize the regulatory bang for the buck.

Many scientists, like myself, are concerned that our techniques aren't good enough and we could miss some very, very major problems if we go to a pure quantitative risk assessment basis.

Mr. **Gore**. Dr. Brandt, do you agree with the last two responses?

Dr. **Brandt**. I agree that risk assessment, quantitative risk assessment, is in an early, very early phase.

However, the more risky a compound or an event is, the easier it is to measure that risk.

Therefore, for the high risk ones, it would be worthwhile as an experiment to start trying to compute quantitative risks as a part of the process, but I am not advocating that the results ought to be the basis for a regulatory decision.

Mr. **Gore**. And not as a prerequisite before a decision could be made?

Dr. **Rall**. I think that we are not in a position to use it as a prerequisite.

Mr. **Gore**. What about the second response that we ought to greatly increase that percentage which is now devoted to methods development?

Dr. **Brandt**. Yes, I agree with that.

Mr. **Gore**. Anybody disagree with that on our panel?

Dr. **DeVita**. The only comment I would make is that it is not always easy to increase the percentage. That is not as programable as doing a certain number of tests. There you are buying ideas many times.

So, as long as the ideas are coming in, you can buy them, but they are not coming in with the same volume as the chemicals are there to test.

Mr. **Gore**. Dr. DeVita, Dr. Hart, Dr. Millar, from the standpoint of advancing the state of the science in toxicology, in your opinions what has the national toxicology program accomplished that would not otherwise have been achieved by the research agencies now contributing resources to the NTP structure?

We would like to start with you, Dr. Hart.

Dr. **Hart**. I think the major thing the NTP has done or accomplished is the fact that it has brought together the various heads of the different agencies to discuss mutual problems, goals, programs.

This communication has helped prevent gaps or overlaps in these programs and thereby has permitted a better utilization of the funds which we have available for toxicological research.

In this regard, what this has meant in many ways is that at NCTR, which has only about 40 percent of its funds allocated to testing with the rest allocated to methods development can pursue its mission of toxicological research while the NTP pursues its mission of compound evaluation.

We mentioned earlier, when we talked about mission statements for NCTR, this is the reason why NCTR was established. We are well aware that we have certain types of methods that we have to develop in order to do better risk assessment.

One of the first things I did, for example, after we had the program moving at NCTR, was to go over to NTP and discuss with Dr. Rall and Dr. Hoel and some of the other scientists there the development of in vivo biomarkers for toxicity. These methods, we believe, will be much more predictive and hopefully have the potential for extrapolating animal data to human health effects.

This might not have happened if there had not been an NTP, which attempts to generate this type of coordination and interaction.

Mr. GORE. Dr. DeVita?

Dr. DEVITA. I think the Cancer Institute deserves a lot of credit, if I may say so, for developing a bioassay program when one did not exist.

It never developed this program to be a source of information for the regulatory agencies. Therefore, when suddenly there was pressure for us to provide routine information, we were not able nor properly constructed to do that.

I think the creation of the NTP created an entity that now provides that kind of information. We can talk about the kinds of things that we have been talking about here, such as risk assessment on a routine basis. To me that advantage, plus bringing all the agencies together so that we understand each other and the needs of the regulatory agencies, has been a major accomplishment.

One other thing, I believe that the prechronic phase testing that the NTP is doing before they go into the long-term bioassays is probably already providing information that will make the end points on carcinogenicity more useful for risk assessment in the future.

I think the program has been refined. We like to think we started it, even though we didn't know why we were starting it except to see whether animals could be predictors of cancer. That was an issue back then, what kinds of animals and so forth. I think now it has taken the shape it should have taken—that is, one place where people can turn to get all of this information.

Mr. MILLAR. We have particularly appreciated the coordination that is embodied in NTP. Our scientists have very much profited by their associations with scientists in other agencies and by becoming aware of what these other scientists are doing.

I think there has been an increase in the number of compounds of occupational significance which have been tested as a consequence of the program, and an increase in the amount of testing for effects other than carcinogenicity.

Mr. GORE. Well, very good.

Dr. Brandt, in view of the evident success of this program, can we apply this organizational experiment to other settings within the Federal Government?

Dr. BRANDT. I am sure there must be another place to apply it, but quite frankly I can't put my finger on it.

Mr. GORE. What about epidemiology?

Dr. **Brandt**. At this point in time, I think that would be difficult to do, principally because you are dealing with both acute and chronic problems that are hard to bring together.

With respect to epidemiology, developing the very same model now toward training of epidemiologists. We now have a public health servicewide training program, outside of the epidemiology intelligence service, in which trainees spend time in all of the agencies. They are supported by all the agencies, funds are pooled, their educational program is PHS wide.

Whether we could do epidemiology—all the epidemiology throughout the Public Health Service that way, I would be hard pressed to say.

As you know, we separate the epidemiological intelligence service, which deals with acute problems like legionnaires' disease and toxic shock from the chronic epidemiology or the more long-term epidemiology which is done principally in the NIH.

Mr. **Gore**. You know, there is going to be in my judgment a terrific upsurge in public demand for epidemiology in this country in the next few years.

Dr. **Brandt**. Yes.

Mr. **Gore**. The evidence is already all around us.

A Senator the other day was demanding an epidemiological study in Nevada. The same day I got a letter from a colleague from New York who was trying to devise a way to get an epidemiolgical study done there.

I have had contact from Tennesseans who want an epidemiological study done in a city there.

As more information is made public about environmental risks, about risks associated with drugs, with chemical exposure, and the shortcomings of bioassays and the other tests done become better known, the demand for epidemiology is just bound to increase.

It seems to me that this organizational model which has been so successful might well be applied to epidemiology, and I would hope that HHS could consider that.

Dr. Rall, do you have any opinion on that?

Dr. **Rall**. I am not sure there is the institutional focus for chronic disease epidemiology, which is basically what we are talking about, in the Public Health Service the way there was in NIEHS a focus for toxicological studies.

In many ways it seemed to me that the presence of NIEHS with a main focus on toxicology, with supportive training, supportive grants, and so forth, is what made addition of the targeted testing and test development program feasible, but we do not have that model that I am aware of in the Public Health Service for epidemiology.

Dr. **DeVita**. May I make a comment?

The creation of a program like the NTP depends upon how sharply focused your end point is. It seems to me for epidemiology we are going in the opposite direction; that we are trying to move the epidemiologist closer to the laboratory scientists in their respective fields.

So in the cancer field we are trying to have the epidemiologists work more closely with the people doing the metabolic carcinogenesis studies, for example. That is becoming an increasingly important role.

To put all epidemiologists in one pot assumes they are all generalists. That is not the case.

I would think it is just the opposite for epidemiologists. We certainly do need more epidemiologists. That is a problem for us all.

Mr. GORE. I would like to recognize counsel for the minority, Mr. Clement.

Mr. CLEMENT. Thank you, Mr. Chairman.

Gentlemen, several of you testified earlier today that formaldehyde is a potential human carcinogen. I would like to ask each of you the following question: Can you at this time state categorically that formaldehyde is, in fact, a human carcinogen? Dr. Rall?

Dr. RALL. No, we cannot. I think we may never be able to because everyone is exposed to formaldehyde.

Mr. CLEMENT. Dr. DeVita?

Dr. RALL. It is possible we may never be able to develop an adequate epidemiological study.

Dr. DEVITA. To go absolutely by our own criteria, we require demonstration of its carcinogenicity in two species at the moment. I am aware of it only in one. That is why the qualifier "potential" is added.

Mr. CLEMENT. Dr. Hart?

Dr. HART. I agree with the other gentlemen.

Mr. CLEMENT. You also say no?

Dr. HART. Yes.

Mr. CLEMENT. Dr. Millar?

Dr. MILLAR. You have to use the qualifier "potential."

Mr. CLEMENT. You would also say no?

Dr. MILLAR. Yes.

Mr. CLEMENT. Can you, as scientists remembering what happened with the Government experience on nitrites and on saccharin, understand that OSHA as a regulatory agency might choose not to establish an agency policy on a chemical until the scientific community can say categorically that a chemical is a human carcinogen as differentiated from your view as scientists?

Is that understandable to you, Dr. Rall?

Dr. RALL. Yes, but what you are proposing is a revolution in regulation.

Mr. CLEMENT. I am not proposing anything, Dr. Rall. I am asking you, can you, as a scientist——

Mr. GORE. Let's let the witness respond to the question.

Dr. RALL. If the evidence must be—speaking as a scientist now—that it is a human carcinogen, very few compounds could be regulated.

There are only about 20 compounds that are known to be carcinogenic in man. These are very, very difficult studies to do, extraordinarily difficult, time consuming, expensive, and it really changes the whole ground rules.

I don't know whether you realize that.

Mr. CLEMENT. Are there reputable scientists based on the information available today that has been published in the scientific literature and that has been subject to peer review who believe that formaldehyde might not be a human carcinogen?

Dr. RALL. Oh, I am sure. The scientific community is very large. [Laughter.]

Mr. CLEMENT. Dr. DeVita, would you agree there is room for disagreement on the question of whether or not formaldehyde is a human carcinogen?

Dr. DEVITA. I can see why an agency might require the absolute criteria as we define it as absolute criteria.

I think Dr. Rall's point is also a good one, but a different one. If we say tests in two species are required and we don't have two species, I can see where one might waiver until we have two species.

Mr. CLEMENT. Would you answer the question, however? Is there room for disagreement within the scientific community as to whether or not formaldehyde is, in fact, a human carcinogen?

Dr. DEVITA. I am sure there is. I don't know of anybody at the moment who has taken the position that it isn't, but I wouldn't be surprised if you could find somebody.

Mr. CLEMENT. Thank you.

Dr. Hart, would you agree there is room for disagreement?

Dr. HART. There is always room for disagreement in the scientific community by definition.

Mr. CLEMENT. Dr. Millar, would you agree with that also?

Dr. MILLAR. I would agree with what Dr. DeVita said about it.

Mr. CLEMENT. I believe he said there was room for disagreement.

Thank you, Mr. Chairman. No further questions.

Mr. GORE. I think Dr. DeVita said he wasn't familiar with anyone who had taken that position.

Mr. CLEMENT. That is true.

Mr. GORE. In fact, the Formaldehyde Institute participated in this Federal Formaldehyde Panel, is that correct, Dr. Rall?

Excuse me, in the CIIT, is that correct?

Dr. RALL. I don't know. I just don't know.

Mr. GORE. Will all of you agree to answer further questions in writing for the record?

Dr. RALL. Yes.

Dr. BRANDT. Yes.

Mr. GORE. We have a number of other questions about this. I don't think it will take you a lot of time.

We are trying to do a definitive job of assessing this program, but we will defer other questions until that time and in that manner.

Let me close this first day of the 2-day hearing by thanking all of our distinguished witnesses. We are honored to have such an abundance of talent all at the same time here at the witness table.

We appreciate your time and your responses. I wish to congratulate all of you who have made this experiment work as well as it has. It is a joy to conduct an oversight hearing where the work is going as well as this work evidently is, and the shortcomings that we attempt to identify are as few comparatively as they are in this area.

So I congratulate you for your work.

We will reconvene tomorrow at 2 p.m. in this room.

[Whereupon, at 5 p.m., the subcommittee was adjourned, to reconvene at 2 p.m., Thursday, July 16, 1981.]

[Additional material requested by Mr. Gore follows:]

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

July 20, 1981

NATIONAL INSTITUTE OF
ENVIRONMENTAL HEALTH SCIENCES
P.O. BOX 12233
RESEARCH TRIANGLE PARK, N.C. 27709

Congressman Albert Gore, Jr.
Longworth House Office Building
Room 1131
Washington, DC 20515

Dear Congressman Gore:

Drs. Rall and Moore asked me to respond to your request at the July 15 hearing of the "Investigation and Oversite Subcommittee of the House Committee on Science and Technology" for copies of our asbestos studies in hamsters. Both reports are enclosed.

I would like to point out that these are "Draft" reports and are subject to revision. Some of the numbers in the "Analysis of Feed Table" may be changed. However, these and other changes will be of a minor nature and will not affect the outcome of the study in my opinion.

I hope this material responds fully to your request. Should you require further information do not hesitate to contact me.

Sincerely,

E.E. McConnell, D.V.M.
Chief, Chemical Pathology
National Toxicology Program

cc:
Dr. D.P. Rall
Dr. J.A. Moore

DRAFT

NTP Technical Report

on the

# CARCINOGENESIS BIOASSAY
of
AMOSITE ASBESTOS
in Syrian Golden Hamsters

(CAS RN 12172-73-5)

DRAFT

DRAFT

Carcinogenesis Testing Program
NATIONAL TOXICOLOGY PROGRAM
P. O. Box 12233
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North Carolina 27709

NOTICE

This is not a final report Until this DRAFT is reviewed and approved by the Technical Reports Review Subcommittee of the NTP Board of Scientific Counselors, this DRAFT does not represent the official position of the National Toxicology Program.

23 June 1981
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Public Health Service
Department of Health and Human Services

Copies of this DRAFT Report are printed only for the purpose of scientific peer-review and are not available for general distribution. Three to six months after the peer-review meeting, FINAL Reports are made available from the National Technical Information Service, Springfield, VA 22161 (703-557-4650).

Comments and questions about the DRAFT National Toxicology Program Technical Report on the Carcinogenesis Bioassay of AMOSITE ASBESTOS in Syrian Golden Hamsters should be directed to Dr. E. E. McConnell, National Toxicology Program, P. O. Box 12233, Research Triangle Park, NC 27709 (919-541-3440).

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## CARCINOGENESIS BIOASSAY OF AMOSITE ASBESTOS

### Abstract

A carcinogenesis bioassay of amosite asbestos was conducted using male and female Syrian golden hamsters. The asbestos was administered at the rate of 1% in pelleted diet for the entire lifetime of the hamsters starting with the mothers of the test animals. The control groups consisted of 127 male and 126 female hamsters and the amosite groups contained 252 male and 254 female animals. There was no adverse effect of amosite asbestos on body weight gain or survival. Neither of the amosite groups showed an increased rate of neoplasia in any organ or tissue compared to the control groups.

Under the conditions of this bioassay, the ingestion of amosite asbestos was not carcinogenic in male and female Syrian golden hamsters.

# CARCINOGENESIS BIOASSAY OF

# AMOSITE ASBESTOS
in Syrian Golden Hamsters

## FOREWORD

This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. A negative result, in which the test animals do not have a greater incidence of cancer than control animals, does not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. A positive result demonstrates that the test chemical is carcinogenic for animals under the conditions of the test and indicates that exposure to the chemical could pose a potential hazard to humans. The actual determination of the risk to humans from chemicals found to be carcinogenic in animals requires a wider analysis and extends beyond the purview of this study.

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## I. INTRODUCTION

The term asbestos has a commercial industrial derivation limited to naturally occurring fibrous minerals of the serpentine or amphibole series. Chrysotile is the only asbestos in the serpentine series whereas the amphibole series is represented by crocidolite, amosite, actinolite, tremolite, and anthophyllite. The essential characteristic of asbestos is its fibrous nature. The gross fibers which are visible to the naked eye are actually bundles of much finer fibrils that are submicroscopic in size.

Studies during the past 25 years have clearly established an association between occupational exposure to asbestos and increased risk of cancer. Human studies have shown that increased tumor risk is associated with chrysotile, amosite and anthophyllite exposure; animal studies also implicate crocidolite.

Lung cancer and mesothelioma are neoplasms most frequently observed in people exposed to asbestos with the latter tumor perhaps unique in its association with these fibers. A modest increase in the incidence of gastrointestinal tumors has also been observed among asbestos insulation workers, miners, and factory workers.

Excellent reviews of the carcinogenic and public health effects associated with asbestos are those by Selikoff (1980), by the Environmental Protection Agency (1980), by Selikoff and Hammond (1979), and by the International Agency for Research on Cancer, WHO (1977).

It is plausible to speculate that the increased incidence of gastrointestinal cancer and possibly peritoneal mesothelioma in occupationally exposed populations may be a consequence of direct fiber ingestion or ingestion of inhaled fibers cleared from the nasal or tracheobronchus portions of the respiratory system by mucociliary processes.

Large portions of the population ingest asbestos through consumption of food and water. Analysis of water samples from 365 cities found 45% to have detectable levels of asbestos (Millette, 1979). Fortyone cities had asbestos concentrations in water that exceeded 10 million fibers per liter. Asbestos or asbestos-like fibers may gain access to water supplies as a result of mining (Lake Superior), presence of natural serpentine or amphibole deposits in their water sheds (Seattle, Washington, San Francisco, California), or under certain conditions through the use of asbestoscement pipe by municipal water supplies (EPA, 1980). In the latter instance erosion of fibers is associated with the "aggressiveness" of the water, a term representing a mathematical expression of pH, alkalinity, and calcium content. It was estimated that 68.5% of U.S. water systems utilize water that is potentially capable of eroding asbestoscement pipe (EPA, 1980).

Furthermore, beers and wines could contain asbestos, possibly as a consequence of the use of asbestos filters in the preparation of these products (Cunningham and Pontefract, 1971). The ingestion of rice treated with asbestos containing talc has been hypothesized to be associated with an increased incidence of stomach cancer (Merliss, 1971a and 1971b).

A number of studies have provided evidence that ingestion of asbestos in either food or water can result in the migration of asbestos fibers through the gastrointestinal mucosa and distant organ sites in humans (Carter and Taylor, 1980), rats (Cunningham, et al., 1977), and baboons (Storeygard and Brown, 1977). Electron microscopic studies confirmed the presence of amphibole mineral fibers in the urine of people who ingested water containing these fibers (Cook and Olso, 1979).

Harrington, et al., (1978) failed to detect an association between the use of asbestos cement pipe for municipal water supplies and the incidence of gastrointestinal cancer. In a study of cancer incidence in the San Francisco Bay area, (Kanarek, et al., 1980) reported a statistically significant trend for the incidence of several cancer types including stomach, gallbladder, esophageal and peritoneal cancer when analyzing census tracts on a gradient of low to high asbestos content in municipal water. In subsequent studies, Cooper, et al., (1979) confirmed the association between asbestos levels in San Francisco Bay area drinking water and cancer of the digestive tract.

Studies in animals have shown that the inhalation of asbestos can produce lung carcinoma and mesothelioma in the pleural cavity. Intrapleural, intratracheal and intraperitoneal injection of asbestos will also produce neoplasia in several species of laboratory animals. A review of the results of these animal studies is given by Levine (1978).

Asbestos (chrysotile, amosite and crocidolite) has been shown to be cytotoxic *in vitro* to human embryonic intestine and mouse epithelial-like colon derived cells and rat liver epithelial cells (Reiss et al., 1979). However, chrysotile asbestos was far more cytotoxic than the amphibole fibers and effects were more pronounced in the intestine derived cells than those from the liver. Asbestos was also found to be cytotoxic to Syrian hamster peritoneal macrophages (Bey and Harrington, 1971).

Reiss et al. (1979) showed that the above three forms of asbestos were not mutagenic using the HGPRT locus/resistance to 6-thioguanine assay system. In addition, no mutagenic activity was demonstrated using chrysotile, amosite or crocidolite asbestos in *E. coli* or *S. typhimurium* systems (Chamberlain and Tarmy, 1977). Therefore, it can be concluded that asbestos is not genotoxic but rather an epigenetic carcinogen of the solid state type (Weisburger and Williams, 1979).

In 1973 the National Institute of Environmental Health Sciences and the Environmental Protection Agency cosponsored a symposium on the possible biological effects of ingested asbestos (EHP, 1974). A concensus conclusion of that conference was that a paucity of data existed concerning the effects of ingested asbestos and that specific research was needed.

A Subcommittee of the DHEW Committee to Coordinate Toxicology and Related Programs subsequently reviewed existing data, and prepared a draft research protocol that the Committee felt was responsive to the major public health consensus. The protocol was widely distributed within and outside the government for comment. Based on the comments received, a revised final protocol was developed which required long-term animal toxicology studies whereby ingestion of several forms of asbestos would be evaluated for carcinogenic effect. The forms of asbestos to be studied included chrysotile (a serpentine asbestos), amosite and crocidolite (representative of amphibole asbestos), and a nonfibrous tremolite which contained low levels of asbestiform fibers.

All materials were to be tested in the Fischer 344 strain of rat whereas two forms of asbestos were to be tested in hamsters. All studies were to encompass the lifetime of the animal, defined as the period from which the animal commences eating solid food until death.

This report represents the results of those studies undertaken to determine the effects of amosite asbestos in the diet fed to Syrian golden hamsters.

## MATERIALS AND METHODS

### Test Materials

Asbestos is a general term applied to certain natural mineral silicates when they appear in a fibrous form. Amosite is a fibrous member of the amphibole mineral group, its chemical structure is (FeMg) $SiO_3 \cdot 1\text{-}5\%\ H_2O$.

The amosite sample identified as S-33 was purchased by the Bureau of Mines from the Atlas Asbestos Company, Montreal, Quebec, Canada. This material is from a mine in the area known as Penge, in the Transvaal, Republic of South Africa. Not a proper mineral name, amosite is a term used to describe the material from asbestos mines in South Africa.

To develop better homogeneity of the sample the amosite was processed by a single pass through an air jet mill. The high abrasive action of amosite resulted in erosion of the steel surfaces of the mill resulting in the chromium content of the milled product increasing from 90 to 170 ppm.

The 1200 pounds of milled amosite was packaged as 20 pound lots in virgin fiberboard drums and stored with other forms of asbestos in a special warehouse at Research Triangle Park, North Carolina. Each drum received a color marking unique to the asbestos type. Random samples of various drums determined that the amosite was of uniform homogeneity.

The homogenicity of the samples and the physical and chemical properties of the materials were extensively characterized by the Bureau of Mines, U.S. Department of the Interior, (Supt. of Documents No. 1 28.23:8452); and the Fine Particle Laboratories, Illinois Institute of Technology Research Institute, Chicago, Illinois (Special Report and Addendum on project L6085, contract Nol-ES-5-3157). Copies of these reports are available upon request from the National Toxicology Program.

Selected chemical and physical properties of amosite asbestos are given below.

### Mineralogical Composition

Grunerite asbestos was detected at a volume percent abundance of about 94, actinolite asbestos about 5; minor amounts of biotite, siderite, plagioclase, ziosite, glass, opagues, and quartz.

### Fiber Characteristics

| | |
|---|---|
| Surface area ($m^2/g$) | 4.13 |
| Density (g/cm) | 3.35 ± .026 SD |
| Measurements, Transmission Electron Microscopy | |
| fiber count/gm | $0.3466\text{X}10^{10}$ |
| median length (μm) | 4.37 |
| range of length | 0.85 - 995 |
| median diameter | 0.72 |
| range of diameter | 0.064 - 12.4 |
| median fiber aspect ratio (l/d) | 6.4248 |

### Chemical - Instrumental Asbestos (Expressed as weight percent)

| | |
|---|---|
| $Al_2O_3$ | .42 |
| CaO | .48 |
| FeO | 34.61 |
| $Fe_2O_3$ | 2.24 |
| MgO | 6.22 |
| $K_2O$ | .30 |
| $SiO_2$ | 50.36 |
| $Na_2O$ | .03 |
| MnO | 2.66 |
| $Cr_2O_3$ | .03 |
| NiO | .01 |
| $Co_2$ | .88 |
| $H_2O^-$ | .15 |

| | |
|---|---|
| $H_2O^+$ | 2.30 |
| Benzene extracted organics | .021 |

Test Diets

The diet used was NIH31 open formula rodent diet prepared by Zeigler Brothers Inc., Gardners, PA. The test diet differed from the control diet through the incorporation to a level of 1% by weight of the appropriate amosite asbestos. Pilot studies determined that homogeneous mixing of asbestos in the diet occurred in a 55 cu. ft. Patterson Kelly "V" blender if the blender was loaded by alternate layering of feed and asbestos. All feed was pelleted using a SproutWaldron pelleter; the pellets were of oval configuration 3/8" X 3/4" size. Pelleted feed was packaged in 25 pound aliquots using standard paperfeed bags which were color coded to minimize the occurrence of feeding errors at the test laboratory.

Each lot of blended feed was analyzed for asbestos concentration; the results of these analyses are given in Appendix A.

Source and Specifications of Test Animals

Four groups (3 chrysotile and 1 amosite) of disease free, mated female outbred Syrian golden hamsters were obtained over a period of 20 weeks in 1977 from Charles River Lakeview Laboratories, New Field, NJ. The hamsters had been mated 6 days prior to shipping.

Animal Maintenance (Table 1)

Upon arrival the mated female hamsters were weighed and sorted into weight ranges. They were then distributed randomly between control and treatment groups which were housed in separate rooms. Each dam was placed in an individual cage with filter top in their respective rooms. Treated or control food was provided ad libitum by placing on the floor of each cage. Water was provided ad

Table 1. Materials and Methods for Animal Maintenance

| Item | Manufacturer | Specifications | Frequency of Change or Cleaning |
|---|---|---|---|
| Cages | Maryland Plastics<br>New York, NY | Econo-Cage<br>Polycarbonate<br>19" x 10 1/2" x 8" | 1X/wk |
| Racks | Bussy Products | Stainless Steel<br>20 cages/rack | 1X/month |
| Bedding | Ab-sorb-Dri, Inc.<br>Rochelle Park, NJ | Hardwood<br>50 lb/bag | 1X/wk |
| Cage tops | Able Molded Plastics, Inc.<br>Chicago, IL | GE Lexan<br>Polycarbonate | 1X/month |
| Cage Filter | Associated Air Filter Co.<br>Rosemont, IL | 3" Diameter cut from FG50 Filter Mats | 1X/month |
| Metal Holder for Cage Filter | C.D. Cash Manufacturing<br>Chicago, IL | Outer shell with screen and baffle<br>Inner shell with screen | 1X/month |
| Snap Ring for Holder | Keats-Lorenz Spring Co.<br>Chicago, IL | Phosphor Bronz<br>Spring tempered | 1X/month |
| Feed dish with Metal Lips | W. Braun Company<br>Chicago, IL | 16 oz. Opal or clear glass jars | 1X/wk |
| Feed Follower | Unifab Corp.<br>Kalamazoo, MI. | Stainless Steel with 7 holes | 1X/wk |
| Water Bottles | Continental Glass Co.<br>Chicago, IL | Pint Flint glass | 1X/wk |

Table 1 (Continued). Materials and Methods for Animal Maintenance

| Item | Manufacturer | Specifications | Frequency of Change or Cleaning |
|---|---|---|---|
| Watering Tube | Wahmann Mfg. Co.<br>Timonium, MD | Stainless Steel<br>5/16" OD 7 1/2" length with 120° bend 1 1/2" from bottle | 1X/wk |
| Feed | Zeigler Brothers<br>Gardners, PA | NIH 31 Diet<br>25 or 50 lb bags | 1X/wk |
| Cage & Bottle Washer | Blakeslee<br>Cicero, IL | Tunnel Wash | Daily/Monthly Check Maintenance |
| Autoclaves | American Sterilizer | Models<br>1) Medallion<br>2) RSP (Vacumatic S) | Bimonthly Maintenance |
| Washing Compounds | Economics Labs, Inc.<br>St. Paul, MN | Spearhead<br>Lime Away | |
| Room Air Filters (Exhaust) | (Roughing Filters)<br>Air Filter Equip. Corp.<br>Chicago, IL. | Amer-Glass Filters<br>Type G filters | |
| | (Bag Housing)<br>Pure Air Filter<br>Chicago, IL | Dri Pak<br>2100 H Class II<br>Absolute Filters<br>Am. Air Filter Astrocel | |
| Rack Washer | Metal Wash Machinery<br>Elizabeth, NJ | Mark V | Daily/Monthly Check Maintenance |

libitum via water bottles. The hamsters were not handled except to change the cages just before the litters were due to be born. Once the litters were born they were left undisturbed until approximately 10 days of age. At this time the cages were changed weekly until the offspring were 4 weeks of age, at which time they were weaned.

At weaning the offspring were weighed individually and separated by sex. The dams were killed at this time. Twenty male and 20 female offspring were removed from the study for endo and ectoparasite examination (see appendix B) to confirm that the test groups were of a desired health status. The lifetime study was composed of 4 groups of hamsters; 127 male and 126 female controls, and 252 male and 254 female treated. The test groups were randomly placed into groups of 3 males or 3 females which were housed in polycarbonate cages for the remainder of the lifetime study. Extra offspring were not discarded at this time in case of sexing errors. Approximately 6 weeks after weaning (10 weeks of age) all missexed hamsters were killed along with their cage mates and were replaced with these alternates which had been treated in an identical manner. The remaining extra hamsters were killed. The experimental design insured that ingestion of asbestos spanned the entire phase of solid food consumption during the lifetime of the animal. Food consumption was not determined because of the hamsters habit of sequestering its feed in the bedding. Control hamsters were housed in separate rooms.

During the test period the temperature was maintained at 22°C ± 2° and the relative humidity ranged from 4080%. To minimize contamination of room air with asbestos, each cage was totally enclosed. Incoming air to the cages was filtered through fiberglass filters while exit air was filtered through a fiberglass roughing filter followed by a bag housing filter. The cage atmosphere was negative to the room and the room was maintained at a slightly negative atmosphere in relation to corridor air. Air flow within the animal rooms was maintained with a minimum of 20 air changes per hour. Fluorescent lighting was provided on a 12 hour per day cycle.

Clinical Examination and Pathology

All hamsters were observed daily for signs of toxicity. Individual animal body weights were recorded weekly for the duration of the study. All animals were allowed to die or were killed in a moribund condition with pentobarbitol sodium. A complete post mortem examination was performed on all animals unless severely cannibalized or autolyzed. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group.

Since the gastrointestinal tract was chosen as one of the target organs prior to the study, it was handled in a slightly different manner than what is normally conducted in standard rodent life time bioassays. The entire esophagus was opened and pinned with the exterior surface adjacent to cardboard prior to placement in fixative. The stomach and cecum were prepared in a similar manner. Two cm lengths of duodenum, ileum and 2 portions of jejunum were placed unopened in fixative so that cross sections of these tissues were available for histopathologic examination. The remaining small intestine was opened and gently washed with saline after which it was carefully examined. Suspect lesions were processed separately and identified individually as to location. The entire colon with anus was opened, examined and pinned similarly to cardboard prior to fixation. The size and location of masses were recorded. Masses greater than 1 mm diameter were removed as separate specimens for processing. After fixation the colon was "carpetrolled" starting at the posterior end with the mucosal surface inward prior to embedding.

All tissues were fixed in 10% neutral buffered formalin, sectioned, and stained with hematoxylin and eosin. Tissues/organs examined microscopically were: tissue masses, the above-mentioned portions of gastrointestinal tract, mesenteric and bronchial lymph nodes, mammary gland, salivary gland, thigh muscle, bone marrow (sternum), nasal cavity with turbinates, larynx, trachea, lungs and bronchi, heart, thyroid, parathyroid, liver, gall bladder, pancreas, spleen, kidneys, adrenal glands, urinary bladder, seminal vesicles/prostate/testes, ovaries/uterus, brain, pituitary gland, eyes and spinal cord.

The pathologic findings of the contractor pathologist were subjected to a quality assurance review by an independent pathology contractor. This review consisted of an examination of all tumors diagnosed by the original pathologist, target organs (gastrointestinal tract) from all animals, all organs from 10% randomly selected hamsters and a tissue count on all animals. Sections from all tumors and any other organ where a discrepancy existed between original and reviewing pathologist were submitted to the NTP Pathology Working Group (NTP/PWG) for subsequent review. All slides where there was a discrepancy in tumor diagnosis between the original pathologist and the NTP/PWG were returned to the original pathologist for reevaluation. The tables shown in this report represent the original pathologists' final diagnosis. Reported separately are cases where the original pathologist did not agree with the NTP/PWG.

## Data Recording and Statistical Methods

The individual animal pathology data on this experiment were recorded in the Carcinogenesis Bioassay Data System. The data elements includes descriptive information on the chemicals, animals, experimental design, clinical observations, survival, and individual pathologic results.

Probabilities of survival were estimated by the productlimit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Differences in survival were evaluated by Cox's (1972) life table method.

As noted earlier, concurrent studies were conducted in this laboratory with another form of asbestos (short range chrysotile and intermediate range chrysotile) using exactly the same protocol (NTP, 1981). Although the results of these studies are not given in this report, the chrysotile controls were included with the amosite control groups for statistical purposes as part of the pooled controls.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

For the statistical analysis of tumor incidence data, two different methods of adjusting for intercurrent mortality were employed. Each used the classical methods for combining contingency tables developed by Mantel and Haenszel (1959).

The first method of analysis assumed that all tumors of a given type were fatal, i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the treated and control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results were then combined by MantelHaenszel methods to obtain an overall probability (P) value. This method of adjusting for intercurrent mortality is Cox's (1972) life table method.

The second method of analysis assumed that all tumors of a given type were "incidental", i.e., they were merely observed at autopsy in animals dying of an unrelated cause. According to this approach, the proportions of animals found to have tumors in treated and control groups were compared in each of five time intervals. For male hamsters these time intervals were 0-52 weeks, 53-78 weeks, 79-92 weeks, 93-103 weeks, and greater than 103 weeks. For female hamsters whose median survival was considerably less than the males, the time intervals were 0-44 weeks, 45-52 weeks, 53-60 weeks, 61-68 weeks and greater than 68 weeks. The denominators of these proportions were the number of animals actually autopsied during the time interval. The individual time interval comparisons were then combined by the previously described methods to obtain a single overall result. (See Peto, et al., 1980).

In addition to these tests, one other statistical analysis was carried out for each primary tumor: the Fisher exact test based on the overall proportion of tumor-bearing animals (Gart, et al., 1979). All reported P values are one sided. Except where noted, the results of the three alternative analyses gave similar results.

## III RESULTS

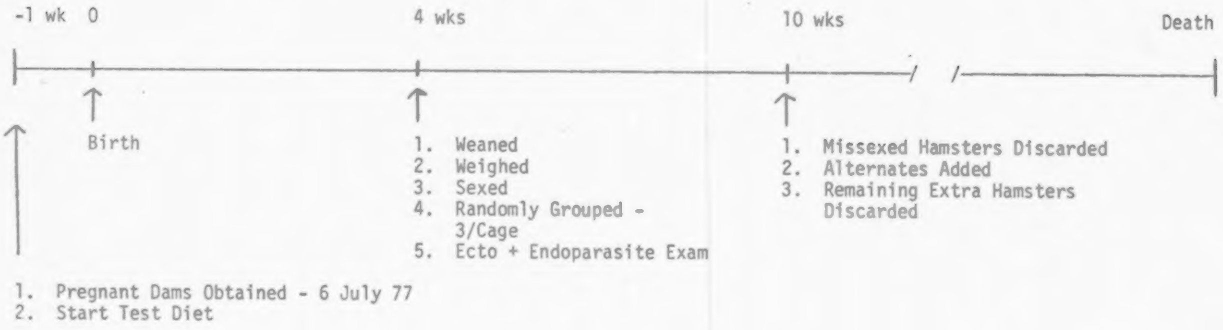
### Establishment of Test Groups

The experiment was designed to evaluate the effects of orally ingested chrysotile asbestos during the entire life of the animal starting from the time it was able to eat solid food. For this reason the mated female hamsters had been on the test diets for approximately 2 weeks when the first litters were born. Ten-15% of the females were either not pregnant, aborted, or their litters died immediately after birth. Several more dams died after showing a prolapsed rectum in the week following birth. The incidences of infertility and neonatal deaths were unrelated to the test diet. The litters were not manipulated or handled during lactation to minimize the chance that the mothers would reject or cannibalize their litters. Many of the pups which died during the nursing period were cannibalized by their mothers. In those pups in which a postmortem examination was possible, the stomachs were typically without food (milk) suggesting maternal rejection, or inability to compete with litter mates. None of these observations were compound related.

Approximately 2% of the offspring in all groups died between weaning and 14 weeks of age due to cage fighting or an enteritis of undetermined origin. Histologically the disease was compatable with the acute form of proliferative ileitis ("wet tail"), a common disease of hamsters. Combinations of cage fighting and enteritis were also observed were also observed but were not compound related. Replacement hamsters were incorporated into the groups (in additional cages) to maintain group sizes until the animals were 10 weeks of age at which time the other extra hamsters were killed (Figure 1).

Body Weight and C

Figure 1. Schedule of Major Events in the Amosite Asbestos Study


-1 wk
0
4 wks
10 wks
Death
Birth
1. Weaned
2. Weighed
3. Sexed
4. Randomly Grouped - 3/Cage
5. Ecto + Endoparasite Exam
1. Missexed Hamsters Discarded
2. Alternates Added
3. Remaining Extra Hamsters Discarded
1. Pregnant Dams Obtained - 6 July 77
2. Start Test Diet

### Body Weight and Clinical Signs

Body weight gain was not adversely affected by the test diets (Figures 2 and 3). In fact amosite asbestos appeared to increase body weight in both male anf female hamsters compared to the temporal control groups. No compound related clinical signs were observed during the entire study. Occasional skin lesions and bite wounds were observed in both sexes but were more apparent in males, and became less of a problem after the hamsters were 20 weeks of age.

### Survival

Survival was not adversely affected by the amosite diet. In fact, the survival rate was significantly ($P<0.01$) better in both male and females on the amosite diet when compared with the corresponding control groups (Figures 4 and 5). The median life span of females (control and treated) was shorter than corresponding groups of males. The median survival of control female group was 55 weeks compared to 81 weeks for control male hamsters in contrast to 60 weeks for treated females and 84 weeks for treated males.

### Pathology

The number of hamsters available for histopathologic examination is shown in Table 2. The majority of animals not included in the pathologic analysis were excluded because of autolysis or cannibalism. Review of the clinical records of hamsters lost to autolysis or cannabalization gave no indication that such animals had neoplasms.

A variety of neoplasms was observed in control (Tables 3 and 4) and amosite exposed hamsters (Tables 5 and 6). The proportions of control male or female hamsters bearing primary tumors were not statistically different among the 4 control groups. Thus, statistical comparisons were made with pooled controls as well as temporal controls. Overall the male hamsters had a slightly higher rate of neoplasia than the females.

Table 2. Disposition of Hamsters from the Amosite Asbestos Feeding Study

| Group | Sex | On Test | Histopathologic Evaluation | Missing | Cannibalized | Autolyzed | Missexed |
|---|---|---|---|---|---|---|---|
| Amosite Control | M | 127 | 122 | 0 | 0 | 4 | 1 |
| | F | 126 | 119 | 1 | 0 | 1 | 5 |
| Amosite | M | 252 | 248 | 0 | 0 | 3 | 1 |
| | F | 254 | 237 | 5 | 0 | 5 | 7 |

Table 3. Incidence of Primary Tumors in Male Hamster Control Groups

| | SR Chrysotile Control | IR Chrysotile Control | DMH and IR Chrysotile Control | Amosite Control |
|---|---|---|---|---|
| Animals with primary tumors | 21/115 (18) | 26/116 (22) | 27/119 (23) | 21/122 (17) |
| Skin or sub. tissue: All tumors | 0/115 (0) | 1/116 (1) | 1/119 (1) | 0/122 (0) |
| Lung or trachea: All tumors | 0/115 (0) | 0/116 (0) | 0/119 (0) | 0/120 (0) |
| Adrenal: Cortical adenoma | 7/115 (6) | 7/115 (6) | 3/117 (3) | 8/119 (7) |
| Cortical carcinoma | 3/115 (3) | 3/115 (3) | 4/117 (4) | 3/119 (3) |
| Pheochromocytoma | 2/115 (2) | 5/115 (4) | 3/117 (3) | 3/119 (3) |
| Other tumors | 0/115 (0) | 3/115 (3) | 2/117 (2) | 1/119 (1) |
| Pancreas: Islet cell adenoma | 2/111 (2) | 7/110 (6) | 8/110 (7) | 3/114 (3) |
| Islet cell carcinoma | 1/111 (1) | 0/110 (0) | 0/110 (0) | 0/114 (0) |
| Thyroid: C-cell adenoma | 3/109 (3) | 3/106 (3) | 0/107 (0) | 1/106 (1) |
| C-cell carcinoma | 1/109 (1) | 1/106 (1) | 0/107 (0) | 1/106 (1) |
| Other | 0/109 (0) | 0/106 (0) | 1/107 (1) | 0/106 (0) |
| Parathyroid: Adenoma | 0/72 (0) | 1/71 (1) | 1/64 (2) | 0/64 (0) |
| G.I. Tract: All tumors | 2/115 (2) | 1/116 (1) | 2/119 (2) | 1/122 (1) |
| Pituitary: All tumors | 0/84 (0) | 0/77 (0) | 0/80 (0) | 0/81 (0) |
| Kidney: All tumors | 0/115 (0) | 2/116 (2) | 1/119 (1) | 1/120 (1) |
| Liver: All tumors | 0/115 (0) | 0/116 (0) | 0/119 (0) | 0/120 (0) |
| Leukemia or malignant lymphoma | 2/115 (2) | 1/116 (1) | 4/119 (4) | 1/122 (1) |
| Hemangioma or Hemangiosarcoma | 0/115 (0) | 0/116 (0) | 3/119 (3) | 2/122 (2) |
| All other tumors | 1/115 (1) | 0/116 (0) | 3/119 (3) | 1/122 (1) |

( ) Percent.

Table 4. Incidence of Primary Tumors in
Female Hamster Control Groups

| | Short Range Chrysotile Control | Intermediate Range Chrysotile Control | DMH & Intermediate Range Chrysotile Control | Amosite Control |
|---|---|---|---|---|
| Animals with primary tumors | 19/114 (17) | 17/119 (14) | 15/120 (12) | 15/119 (13) |
| Skin or sub tissue: All tumors | 0/114 (0) | 0/119 (0) | 0/120 (0) | 0/119 (0) |
| Lung or trachea: All tumors | 0/114 (0) | 0/119 (0) | 0/119 (0) | 0/119 (0) |
| Adrenal: Cortical adenoma | 4/112 (4) | 6/118 (5) | 3/120 (2) | 2/118 (2) |
| Cortical carcinoma | 0/112 (0) | 0/118 (0) | 0/120 (0) | 0/118 (0) |
| Pheochromocytoma | 0/112 (0) | 0/118 (0) | 0/120 (0) | 0/118 (0) |
| Other tumors | 0/112 (0) | 0/118 (0) | 0/120 (0) | 0/118 (0) |
| Pancreas: Islet cell adenoma | 2/109 (2) | 5/116 (4) | 5/116 (4) | 3/115 (3) |
| Islet cell carcinoma | 1/109 (1) | 0/116 (0) | 0/116 (0) | 0/115 (0) |
| Thyroid: C-cell adenoma | 2/107 (2) | 3/115 (3) | 0/112 (0) | 1/106 (1) |
| C-cell carcinoma | 0/107 (0) | 0/115 (0) | 1/112 (1) | 0/106 (0) |
| Other | 2/107 (2) | 0/115 (0) | 0/112 (0) | 0/106 (0) |
| Parathyroid: Adenoma | 3/68 (4) | 1/77 (1) | 1/74 (1) | 1/61 (1) |
| G.I. Tract: All tumors | 1/114 (1) | 2/119 (2) | 1/120 (1) | 1/119 (1) |
| Pituitary: All tumors | 0/77 (0) | 2/67 (3) | 0/62 (0) | 0/79 (0) |
| Kidney: All tumors | 0/114 (0) | 1/119 (1) | 0/120 (0) | 1/119 (0) |
| Liver: All tumors | 0/114 (0) | 0/119 (0) | 0/119 (0) | 0/118 (0) |
| Leukemia or malignant lymphoma | 2/114 (2) | 0/119 (0) | 3/120 (2) | 2/119 (2) |
| Hemangioma or Hemangiosarcoma | 0/114 (0) | 0/119 (0) | 1/120 (1) | 1/119 (1) |
| Uterus: All tumors | 3/113 (3) | 1/119 (1) | 2/120 (2) | 2/119 (2) |
| All other tumors | 3/114 (3) | 0/119 (0) | 1/120 (1) | 2/119 (2) |

(  ) Percent.

Table 5. Incidence of Primary Tumors in
Male Hamsters Administered Amosite

| | Pooled Controls | Amosite Controls | Amosite |
|---|---|---|---|
| Animals with primary tumors | 95/472 (20) | 21/122 (17) | 57/248 (23) |
| Skin or sub tissue: All tumors | 2/472 (<1) | 0/122 (0) | 0/248 (0) |
| Lung or trachea: All tumors | 0/470 (0) | 0/120 (0) | 0/248 (0) |
| Adrenal: Cortical adenoma | 25/466 (5) | 8/119 (7) | 13/246 (5) |
| Cortical carcinoma | 13/466 (3) | 3/119 (3) | 7/246 (3) |
| Pheochromocytoma | 13/466 (3) | 3/119 (3) | 4/246 (2) |
| Other tumors | 6/466 (1) | 1/119 (1) | 2/246 (1) |
| Pancreas: Islet cell adenoma | 20/445 (4) | 3/114 (3) | 11/234 (5) |
| Islet cell carcinoma | 1/445 (<1) | 0/114 (0) | 0/234 (0) |
| Thyroid: C-cell adenoma | 7/428 (2) | 1/106 (1) | 7/221 (3) |
| C-cell carcinoma | 3/428 (1) | 1/106 (1) | 2/221 (1) |
| Other | 1/428 (<1) | 0/106 (0) | 2/221 (1) |
| Parathyroid: Adenoma | 2/271 (1) | 0/64 (0) | 2/150 (1) |
| G.I. Tract: All tumors | 6/472 (1) | 1/122 (1) | 6/248 (2) |
| Pituitary: All tumors | 0/322 (0) | 0/81 (0) | 0/182 (0) |
| Kidney: All tumors | 4/470 (1) | 1/120 (1) | 2/248 (1) |
| Liver: All tumors | 0/470 (0) | 0/120 (0) | 0/247 (0) |
| Leukemia or malignant lymphoma | 8/472 (2) | 1/122 (1) | 5/248 (2) |
| Hemangioma or Hemangiosarcoma | 5/472 (1) | 2/122 (2) | 2/248 (1) |
| All other tumors | 5/472 (1) | 1/122 (1) | 2/248 (1) |

( ) Percent.

Table 6. Incidence of Primary Tumors in
Female Hamsters Administered Amosite

| | Pooled Controls | Amosite Controls | Amosite |
|---|---|---|---|
| Animals with primary tumors | 66/472 (14) | 15/119 (13) | 30/237 (13) |
| Skin or sub tissue: All tumors | 0/472 (0) | 0/119 (0) | 2/237 (1) |
| Lung or trachea: All tumors | 0/471 (0) | 0/119 (0) | 0/234 (0) |
| Adrenal: Cortical adenoma | 15/468 (3) | 2/118 (2) | 6/234 (3) |
| Cortical carcinoma | 0/468 (0) | 0/118 (0) | 0/234 (0) |
| Pheochromocytoma | 0/468 (0) | 0/118 (0) | 2/234 (1) |
| Other tumors | 0/468 (0) | 0/118 (0) | 0/234 (0) |
| Pancreas: Islet cell adenoma | 15/456 (3) | 3/115 (3) | 2/222 (1)* |
| Islet cell carcinoma | 1/456 (<1) | 0/115 (0) | 0/222 (0) |
| Thyroid: C-cell adenoma | 6/440 (1) | 1/106 (1) | 4/215 (2) |
| C-cell carcinoma | 1/440 (<1) | 0/106 (0) | 1/215 (<1) |
| Other | 2/440 (<1) | 0/106 (2) | 0/215 (0) |
| Parathyroid: Adenoma | 6/280 (2) | 1/61 (1) | 1/141 (1) |
| G.I. Tract: All tumors | 5/472 (1) | 1/119 (1) | 4/237 (2) |
| Pituitary: All tumors | 2/285 (1) | 0/79 (0) | 0/149 (0) |
| Kidney: All tumors | 1/472 (<1) | 0/119 (0) | 0/236 (0) |
| Liver: All tumors | 0/472 (0) | 0/118 (0) | 0/234 (0) |
| Leukemia or malignant lymphoma | 7/472 (1) | 2/119 (2) | 3/2236 (1) |
| Hemangioma or Hemangiosarcoma | 2/472 (<1) | 1/119 (1) | 3/237 (1) |
| Uterus: All tumors | 8/471 (2) | 2/119 (2) | 1/236 (<1) |
| All other tumors | 6/472 (1) | 2/119 (2) | 2/237 (1) |

( ) Percent.

* = P<0.05. decrease relative to pooled controls (life table and incidental tumor tests.

Figure 2. Growth Curve of Male Hamsters Administered Amosite in the Diet.

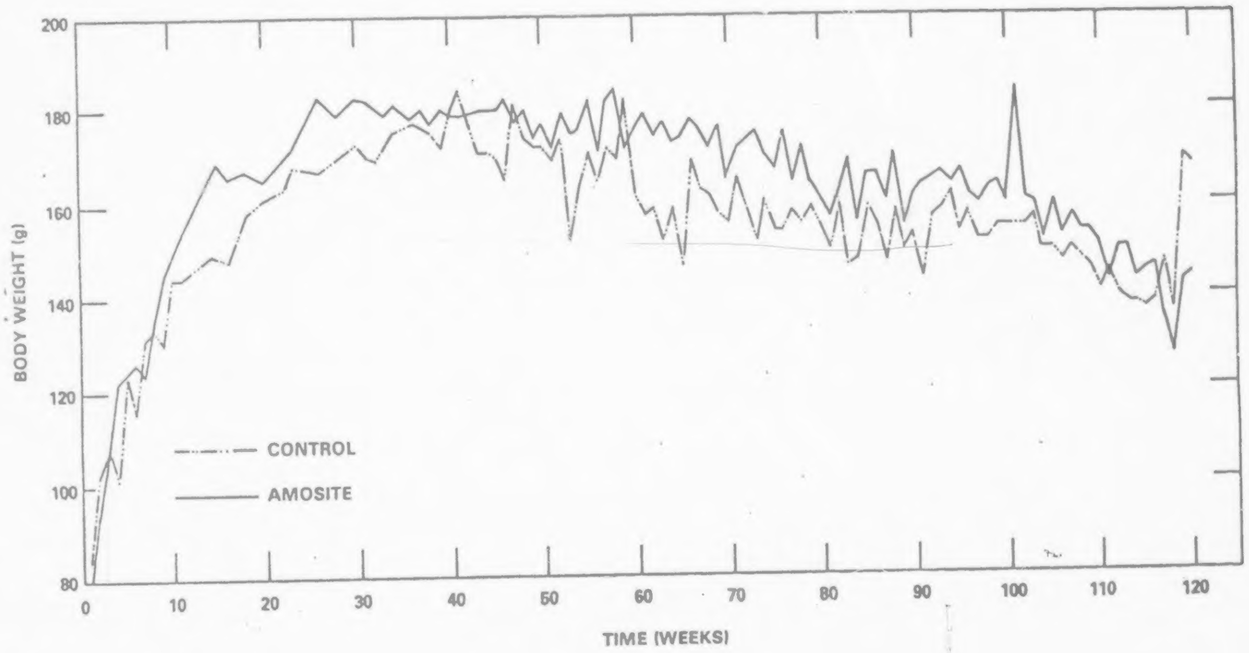


Figure 3. Growth Curve of Female Hamsters Administered Amosite Asbestos in the Diet.

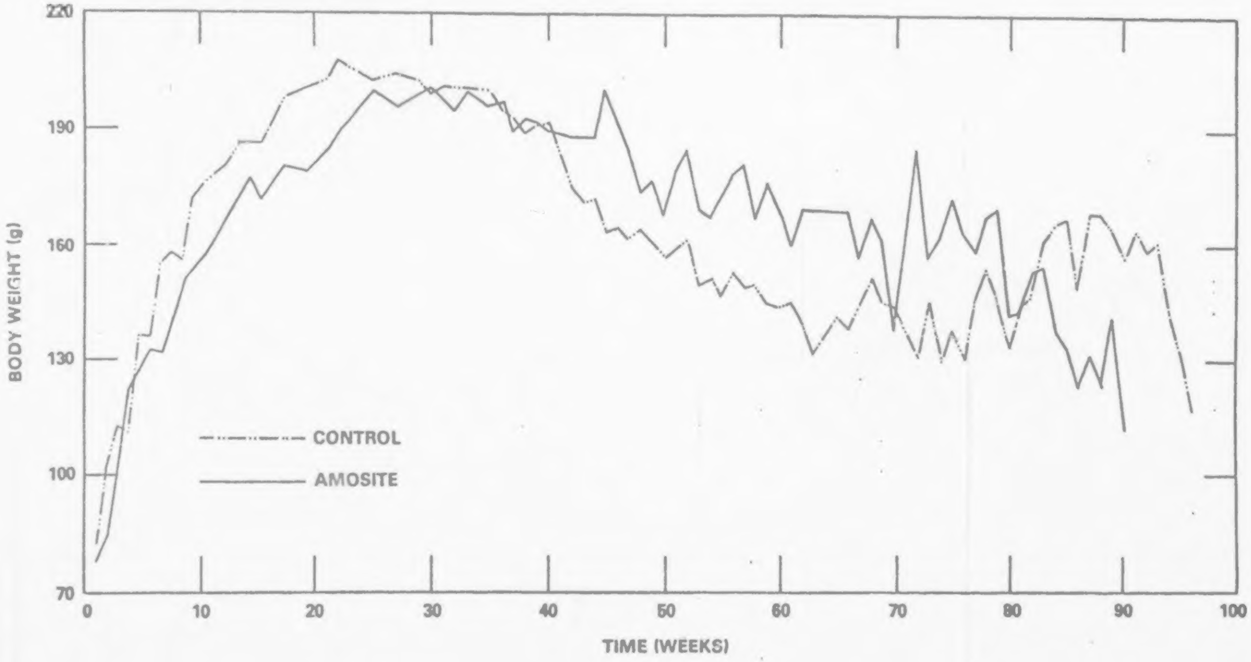


Figure 4. Survival Curve for Male Hamsters Receiving Amosite Asbestos.

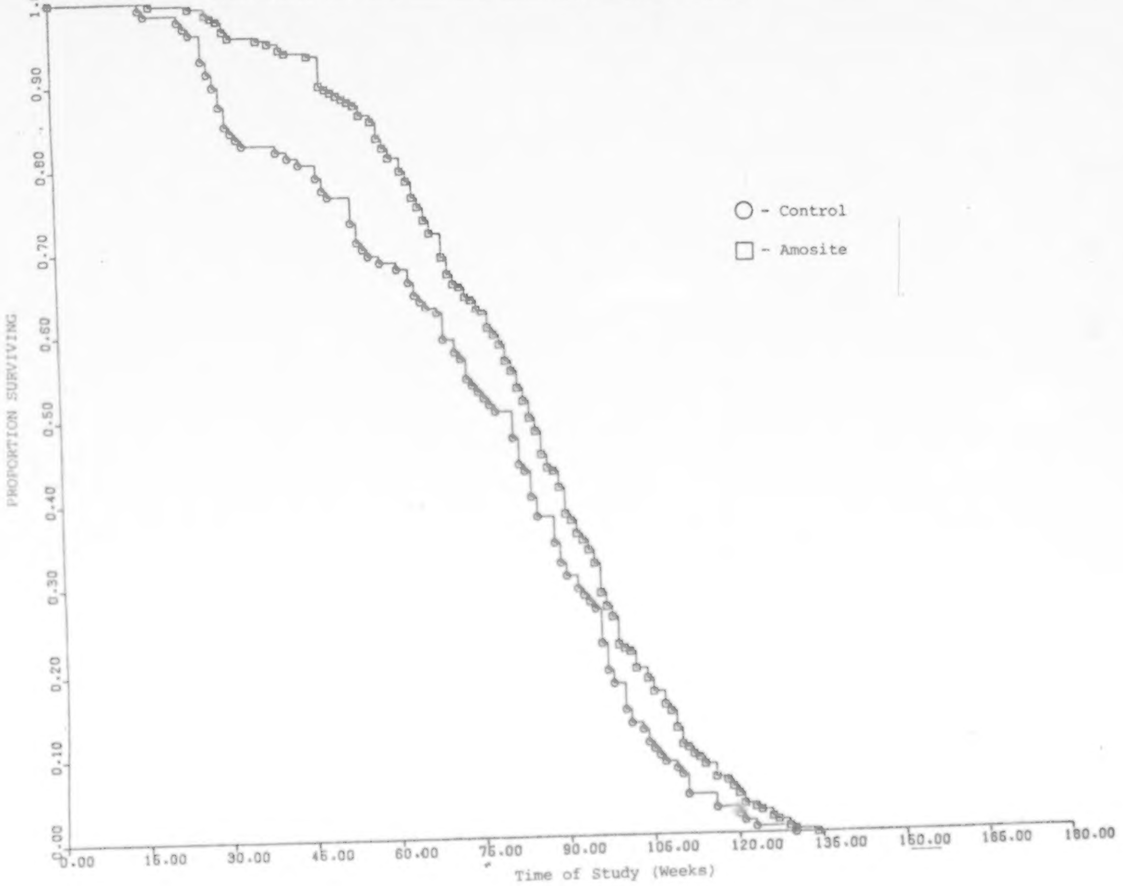
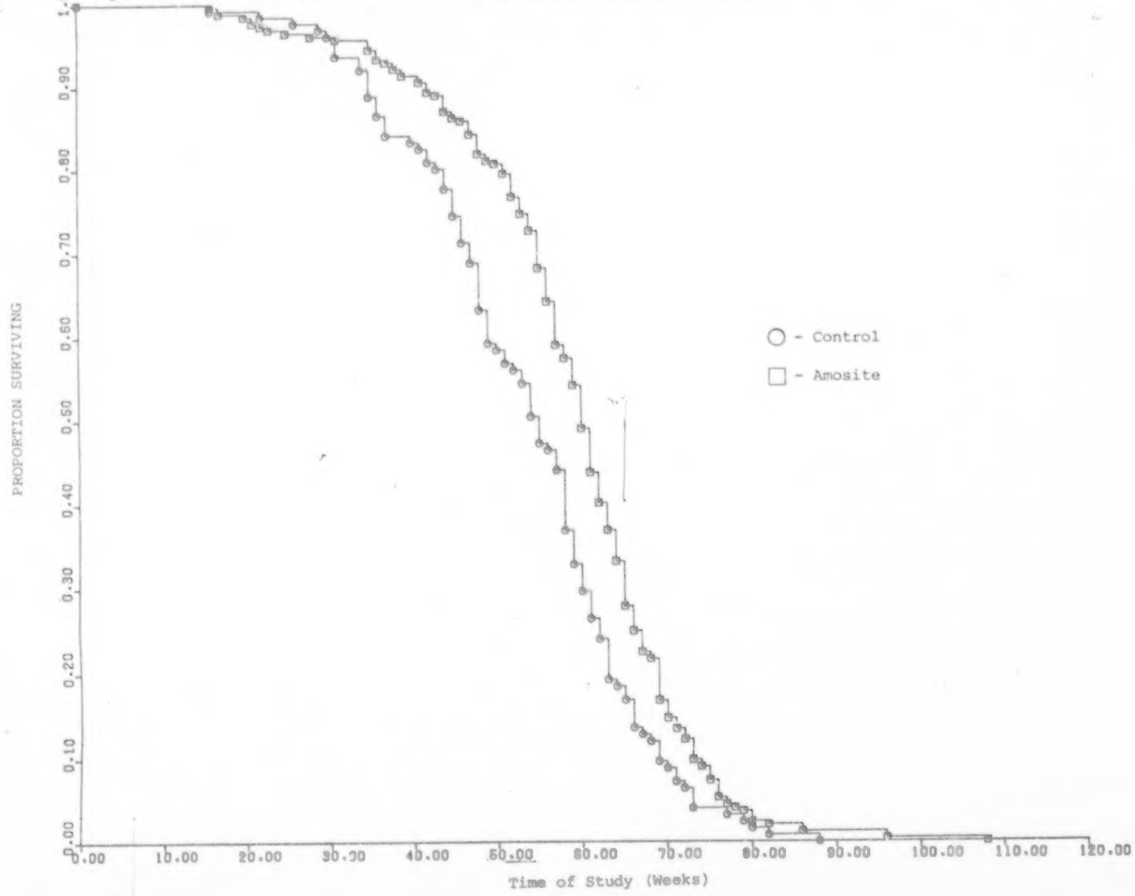


Figure 5. Survival Curve for Female Hamsters Receiving Amosite Asbestos.



Those organs with greater than 4% incidence of neoplasia in treated or control groups were the adrenal gland and pancreas (Islets of Langerhans) and parathyroid. None of the amosite treated groups showed an increased rate of neoplasia compared to their temporal control groups or the pooled control groups, particularly in the proposed target organ, i.e., gastrointestinal tract (Table 7). In fact the only statistically significant ($p<0.05$) difference in tumor incidence observed in this study was a decrease in islet cell adenomas observed in female hamsters receiving amosite (Table 6).

The diagnosis of the NTP Pathology Working Group differed with the original pathologists' interpretation as shown in Table 8. The major diagnostic difference was in regard to whether certain adrenal tumors originated in the medulla or cortex. As shown in Table 8, the PWG diagnoses reduced the number of adrenal medullary tumors and increased the number of adrenal cortical tumors but did not alter the results of the study. The criteria used by the NTP/PWG for the diagnosis of adrenal tumors were those reported by Homburger and Russfield (1970), Matsuyama and Suzuki (1970), and Murthy and Russfield (1966). The criteria were as follows:

Adrenal Cortex:

1. Focal hyperplasia - Proliferation of normal appearing cells resembling those of the zona fasciculata. The cells were of uniform size and morphology and mitotic figures were not observed. These lesions were invariably observed in adrenals showing severe amyloidosis. Another type of hyperplastic lesion encountered was the presence of extracapsular nodules of normal appearing cortical tissue. These were completely encased in a connective tissue capsule.

Table 7. Incidence of Gastrointestinal Tract Tumors in the Amosite Asbestos Study

| | Pooled Controls | | Amosite Controls | | Amosite | |
|---|---|---|---|---|---|---|
| | M | F | M | F | M | F |
| Stomach (no. examined) | (464) | (468) | (120) | (117) | (247) | (236) |
| Squamous cell papilloma | 3 | | 1 | | 4 | |
| Papilloma | | | | | | 3 |
| Small Intestine (no. examined) | (467) | (469) | (120) | (117) | (246) | (236) |
| Adenoma | 1 | | | | | |
| Adenocarcinoma | 1 | | | | | |
| Large Intestine (no. examined) | (464) | (468) | (118) | (116) | (246) | (235) |
| Adenoma | | 1[a] | | | | |
| Papillary adenoma | | | | | 1[a] | |
| Adenocarcinoma | | 1 | | | | |
| Lipoma | | 1 | | | | |
| Rectum (no. examined) | (472) | (272) | (122) | (119) | (248) | (237) |
| Adenoma | 1 | 1[a] | | 1[a] | | |
| Adenomatous polyp | | | | | 1 | |
| Fibroma | | 1 | | | | |
| Squamous cell papilloma | | | | | | |

a = The PWG did not diagnose these lesions as tumors.

Table 8. Comparison of Adrenal Tumor Incidence as Determined by Original Pathologist and NTP Pathology Working Group (PWG)

| | Pooled Controls | Amosite Controls | Amosite |
|---|---|---|---|
| Males (OP) | | | |
| Cortical adenoma | 25/466 (5) | 8/119 (7) | 13/246 (5) |
| Cortical carcinoma | 13/466 (3) | 3/119 (3) | 7/246 (3) |
| Pheochromocytoma | 13/466 (3) | 3/119 (3) | 4/246 (2) |
| Other | 6/466 (1) | 1/119 (1) | 2/246 (1) |
| Males (PWG) | | | |
| Cortical adenoma | 31/466 (7) | 9/119 (8) | 18/246 (7) |
| Cortical carcinoma | 14/466 (3) | 3/119 (3) | 7/246 (3) |
| Pheochromocytoma | 7/466 (2) | 1/119 (1) | 0/246 (0) |
| Other | 2/466 (<1) | 0/119 (0) | 0/246 (0) |
| Females (OP) | | | |
| Cortical adenoma | 15/468 (3) | 2/118 (2) | 6/234 (3) |
| Cortical carcinoma | 0/468 (0) | 0/118 (0) | 0/234 (0) |
| Pheochromocytoma | 0/468 (0) | 0/118 (0) | 2/234 (1) |
| Other | 0/468 (0) | 0/118 (0) | 0/234 (0) |
| Females (PWG) | | | |
| Cortical adenoma | 19/468 (4) | 4/118 (2) | 8/234 (3) |
| Cortical carcinoma | 0/468 (0) | 0/118 (0) | 0/234 (0) |
| Pheochromocytoma | 0/468 (0) | 0/118 (0) | 1/234 (<1) |
| Other | 0/468 (0) | 0/118 (0) | 0/234 (0) |

( ) Percent.

2. Cortical adenoma - There were two types observed. One type was composed of cells resembling the zona fasciculata which were somewhat pheomorphic and were compressing the adjacent parenchyma. The second type contained similar cells but admixed between them were spindle shaped cells which resembled fibroblasts. Mitotic figures were rare. The border of both types of adenomas was well defined but no capsule was evident.
3. Neurolemoma - This tumor was composed of delicate spindloid cells arranged in parallel palasades. It was well circumscribed but nonencapsulated. No mitotic figures were observed. This type of tumor was of much lower incidence than the previously described adenomas.
4. Cortical carcinoma - Carcinomas were composed of cells resembling both types of the above described adenomas. They were differentiated from adenomas based on pleomorphism, nuclear atypia, increased numbers of mititoc figures and invasive growth through the capsule and/or into adjacent blood vessels. Areas of necrosis and hemorrhage were common.

Adrenal Medulla:

1. Hyperplasia - This lesion was characterized by proliferation of normal appearing cells, although an increase in basophilia was sometimes noted. The normal architecture was preserved and the lesion was usually diffuse in nature.
2. Pheochromocytoma - This lesion consisted of a focal nodular proliferation of fairly normal appearing uniform cells although they were often smaller than normal. Mitotic figures were not observed. Growth was by expansion. The borders were distinct and there appeared to be a delicate capsule.

3. Malignant Pheochromocytoma - The major distinguishing characteristics of this neoplasm were nuclear atypia along with invasive growth.

The major difference in terminology between the original pathologist and the NTP/PWG was in regard to the adrenal tumors (benign and malignant) composed of a mixture of spindle shaped cells and eosinophilic hepatoid-like cells. The original pathologist diagnosed these as pheochromocytomas or malignant pheochromocytomas while the PWG called them cortical adenomas or carcinomas.

While this study was not designed to evaluate non-neoplastic disease, noteworthy lesions were observed although none were apparently treatment related and are consistent with what is normally found in aging hamsters. It was the pathologists' opinion that the most important lesion, responsible for many deaths, was generalized amyloidosis. The kidneys were particularly affected by diffuse accumulation of amyloid which replaced glomeruli and infiltrated tubular interstitium to the point where the normal cortical architecture was obliterated. Other organs which showed significant accumulations of amyloid were adrenal gland, liver, spleen and the epithelium of the small intestine. Amyloid within the walls of blood vessels was observed in many tissues.

Many of the livers were cirrhotic, infiltrated with amyloid, and contained large cystic structures filled with a lightly staining proteinaceous fluid. These structures were interpreted as cystic bile ducts and are consistent with what others have termed "retention cysts". At times these cysts were so large and/or numerous that less than half of the livers remained.

Other non-neoplastic lesions that were observed in greater than 5% of the hamsters in any of the experimental groups are as follows:

1. Skin - chronic dermatitis
2. Lung - interstitial pneumonitis
3. Spleen - lymphoid atrophy
4. Lymph node - hyperplasia
5. Heart - atrial thrombosis
6. Stomach (nonglandular) - hyperkeratosis or acanthosis
7. Urinary bladder - hyperplasia
8. Adrenal gland - cortical and medullary hyperplasia
9. Thyroid gland - follicular atrophy
10. Prostate - inflammation
11. Seminal vesicle - inflammation
12. Testes - Seminiferous atrophy and interstitial hyperplasia

None of these lesions was treatment related.

## IV. SUMMARY, COMMENTS, CONCLUSION

The clinicopathologic results in this study showed that the chronic ingestion of 1% amosite asbestos in the diet did not cause any adverse effect on body weight gain and survival. In fact, both weight gain and survival seemed to be enhanced. An explanation for these observations is not apparent.

The ingestion of amosite asbestos over the lifetime of these hamsters did not cause an increase of tumors at any anatomic site when compared to temporal or pooled control groups. In a concurrent study of chrysotile asbestos (NTP, 1981) an apparent increase in adrenal cortical tumors was observed, but this increase was not observed in the amosite study.

Other such studies involving the long term ingestion of asbestos are few. Donham, et al., (1980) reported equivocal results in F344 rats which were fed a diet containing 10% chrysotile for their lifetime. While they did not observe a statistically significant ($p<0.05$) increase in the number of tumors in exposed animals, the authors believed that there was a trend toward increased colon lesions in general, evidence of penetration of asbestos into the colonic mucosa, possible cytotoxicity to colonic tissues and suggested a possible relationship to peritoneal mesothelioma. Another equivocal study is that reported by Gibel, *et al*., (1976) who described an increase in malignant tumors in the lung, kidney, liver and reticuloendothelial system but no increase in intestinal neoplasia in Wistar rats fed asbestos filter material (20 mg/day) for a period of 8-14 months. Cunningham, *et al*., (1977) reported 2 studies in Wistar male rats using 1% chrysotile in the diet; one study of 24 months and one of 30 months. No intestinal tumors were found in the control rats. Negative results were reported by Gross, *et al*., (1974), who fed rats a diet containing 5% chrysotile asbestos for a period of 21 months with no evidence of intestinal neoplasia.

The only oral asbestos study in hamsters was reported by Smith, *et al*., (1980). They exposed groups of 30 male and female hamsters via drinking water for lifetime to amosite asbestos, mine tailings, beach rock and Lake Superior drinking water. They did not observe adverse effects on body weight or survival time in any of the groups. A peritoneal mesothelioma, one pulmonary carcinoma, and two early squamous cell carcinomas of the nonglandular stomach were found in the hamsters exposed to amosite but the incidence was not statistically significant ($p<0.05$). Their other studies were considered negative. They concluded that the study was essentially negative.

Except for the studies of Donham, *et al*., (1980) and Smith, *et al*., (1980), the other studies were conducted with relatively small numbers of animals. Also some were conducted for an insufficient period of time to adequately test the carcinogenic potential of ingested asbestos.

A long term study of amosite asbestos designed to determine the promotor potential of asbestos was reported by Ward, et al., (1980). They exposed 6 week old male F344 rats 3 times per week for 10 weeks to 1 mg amosite asbestos in saline via gavage. Once per week during this same period half of the rats received subcutaneous injections of 7.4 mg/kg azoxymethane (AOM), a known intestinal carcinogen in animals. The rats were allowed to live out their life span or until they reached 94-95 weeks of age at which time they were killed. They reported an intestinal tumor incidence of 66.7% in AOM alone, 77.1% for amosite plus AOM and 32.6% for amosite alone. The authors concluded that while amosite did not significantly add to the incidence of AOM-induced intestinal neoplasia, amosite alone caused a relatively high rate of intestinal neoplasia. However, there was no untreated control group to compare to the treated groups. These results should also be viewed with some suspicion because the authors also reported a 14% incidence of Zymbal gland tumors in the rats exposed to amosite alone. The historical rate of Zymbal gland tumors in the Bioassay Program is 0.34% indicating that this is an extremely rare spontaneous tumor. However AOM is known to induce Zymbal gland tumors with a single dose of 5.1% mg/kg in male F344 rats producing (interestingly) a 14% incidence of tumors in this organ (Ward, 1975); in this study 5.1 mg/kg AOM also caused a 24% incidence of intestinal neoplasia. An appropriate explanation for the incidence of Zymbal gland tumors in the amosite groups would be that they were inadvertently exposed to AOM. If this occurred these hamsters would also be expected to show a high incidence of intestinal neoplasms.

It should be pointed out that this investigation of the carcinogenic potential of ingested asbestos is a two animal specie effort by the National Toxicology Program. While the results in the hamster appear to be negative, a larger bioassay involving more types of asbestos but using essentially the same protocol (1% diet) in rats is currently being evaluated.

## Conclusion

Under the conditions of this bioassay the ingestion of amosite asbestos was not carcinogenic in male and female Syrian golden hamsters.

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## Appendix A

## CALCULATED VALUES OF AMOSITE ASBESTOS IN INDIVIDUAL LOTS OF FEED AS DETERMINED BY MAGNESIUM CONTENT

| Feed Preparation Data | Calculated Asbestos Content (%) |
|---|---|
| 6-77 | 1.004 |
| 8-77 | 1.035 |
| 10-77 | 1.069 |
| | .993 |
| 1-78 | 1.09 |
| 2-78 | .95 |
| 4-78 | 1.13 |
| | 1.12 |
| 6-78 | 1.14 |
| | 1.12 |
| 8-78 | .99 |
| | 1.04 |
| 10-78 | 1.08 |
| | 1.15 |
| 12-78 | 1.20 |
| | 1.00 |
| 3-79 | 1.10 |
| | .97 |
| | .91 |
| 5-79 | 1.34 |
| | .95 |
| 6-79 | .95 |
| | 1.01 |
| 7-79 | 1.06 |
| | 1.11 |
| 10-79 | .99 |
| | .97 |
| 11-79 | 1.18 |
| | 1.14 |
| 12-79 | .86 |
| | .99 |

Appendix A (continued)

| Feed Preparation Data | Calculated Asbestos Content (%) |
|---|---|
| 2-80 | .91 |
|  | .92 |
| 3-80 | .61 |
|  | .67 |
| 4-80 | .98 |
| 6-80 | 1.10 |
|  | 1.34 |

Note: These data represent unverified working values for all lots of feed used in the hamster and rat studies. Final data may show minor numerical variations. A complete description of the atomic absorption methodology utilized in these analyses will be described in the final report.

## Appendix B

### Disease Status of Hamsters Exposed to Amosite Asbestos

| | Control | | Asbestos (Amosite) | |
|---|---|---|---|---|
| Organism | Mother | Offspring | Mother | Offspring |
| *Mycoplasma* spp. | - | - | - | - |
| *Corynebacterium kutscheri* | - | - | - | - |
| *Salmonella* spp. | - | - | - | - |
| *Streptobacillus moniliformis* | - | - | - | - |
| *Streptococcus pneumoniae* | - | - | - | - |
| *Haemobartonella* spp. | - | - | - | - |
| *Encephalitazoon* spp. | - | - | - | - |
| *Aspicularis tetraptera* | - | - | - | - |
| *Syphacia obvelata* | - | - | - | - |
| *Taxoplasma gondii* | - | - | - | - |
| *Hymenolepsis diminuta* or *nana* | - | - | - | - |
| *Trichomonas* spp. | +++ | +++ | +++ | +++ |
| *Hexamita muris* | ++ | ++ | ++ | ++ |
| *Giardia muris* | ++ | ++ | ++ | ++ |
| *Lymphocytic choriomeningitis* | - | - | - | - |
| Mites | - | - | - | - |
| Fleas | - | - | - | - |
| Lice | - | - | - | - |

DRAFT

NTP Technical Report

on the

# CARCINOGENESIS BIOASSAY

of

CHRYSOTILE ASBESTOS

in Syrian Golden Hamsters

(CAS RN 12001-29-5)

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NOTICE

This is not a final report  Until this DRAFT is reviewed and approved by the Technical Reports Review Subcommittee of the NTP Board of Scientific Counselors, this DRAFT does not represent the official position of the National Toxicology Program.

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Comments and questions about the DRAFT National Toxicology Program Technical Report on the Carcinogenesis Bioassay of CHRYSOTILE ASBESTOS in Syrian Golden Hamsters should be directed to Dr. E. E. McConnell, National Toxicology Program, P. O. Box 12233, Research Triangle Park, NC 27709 (919-541-3440).

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# CARCINOGENESIS BIOASSAY OF

# CHRYSOTILE ASBESTOS
in Syrian Golden Hamsters

## FOREWORD

This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. A negative result, in which the test animals do not have a greater incidence of cancer than control animals, does not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. A positive result demonstrates that the test chemical is carcinogenic for animals under the conditions of the test and indicates that exposure to the chemical could pose a potential hazard to humans. The actual determination of the risk to humans from chemicals found to be carcinogenic in animals requires a wider analysis and extends beyond the purview of this study.

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## CARCINOGENESIS BIOASSAY OF CHRYSOTILE ASBESTOS

### ABSTRACT

Bioassays of short range (SR), intermediate range (IR) or intermediate range chrysotile asbestos in combination with the intestinal carcinogen 1,2-dimethylhydrazine dihydrochloride (DMH) were conducted using male and female Syrian golden hamsters. Both forms of chrysotile asbestos were administered at the rate of 1% in pelleted diet for the entire lifetime of the hamsters starting with mothers of the test animals. Group sizes varied from 125 to 253. There was no adverse effect on body weight gain or survival by either form of asbestos or in the combination study. A significant

A significant increase ($p<0.05$) in adrenal cortical tumors was observed in male hamsters exposed to SR and IR chrysotile asbestos and in females treated with IR chrysotile when compared to the pooled control groups. However, statistical significance ($p<0.05$) was lost when compared to temporal control groups.

Under the conditions of this bioassay the ingestion of short range chrysotile and intermediate range chrysotile asbestos was not carcinogenic in male and female Syrian golden hamsters. While there were significant increases in the rates of adrenal cortical adenomas in male and female hamsters exposed to intermediate chrysotile compared to pooled controls, these incidence rates were not significantly different when compared to the temporal control groups. Additionally, the biologic importance of adrenal tumors in the asbence of target organ neoplasia is questionable. The cocarcinogen studies using IR chrysotile and 1,2-dimethylhydrazine dihydrochloride were considered inadequate because there was no increase in intestinal neoplasia in the DMH group.

## I. INTRODUCTION

The term asbestos has a commercial industrial derivation limited to naturally occurring fibrous minerals of the serpentine or amphibole series. Chrysotile is the only asbestos in the serpentine series whereas the amphibole series is represented by crocidolite, amosite, actinolite, tremolite, and anthophyllite. The essential characteristics of asbestos mineral is their fibrous nature. The gross fibers which are visible to the naked eye are actually bundles of much finer fibrils that are submicroscopic in size.

Studies during the past 25 years have clearly established an association between occupational exposure to asbestos and increased risk of cancer. Human studies have shown that increased tumor risk is associated with chrysotile, amosite and anthophyllite exposure; animal studies also implicate crocidolite.

Lung cancer and mesothelioma are neoplasms most frequently observed in people exposed to asbestos with the latter tumor perhaps unique in its association with these fibers. A modest increase in the incidence of gastrointestinal tumors has also been observed among asbestos insulation workers, miners, and factory workers.

Excellent reviews of the carcinogenic and public health effects associated with asbestos are those by Selikoff (1980), by the Environmental Protection Agency (1980), by Selikoff and Hammond (1979), and by the Environmental Agency for Research on Cancer (1977).

It is plausible to speculate that the increased incidence of gastrointestinal cancer and possibly peritoneal mesothelioma in occupationally exposed populations may be a consequence of direct fiber ingestion or ingestion of inhaled fibers cleared from the nasal or tracheobronchus portions of the respiratory system by mucociliary processes.

Large portions of the population ingest asbestos through consumption of food and water. Analysis of water samples from 365 cities found 45% to have detectable levels of asbestos (Millette, 1979). Forty-one cities had asbestos concentrations in water that exceeded 10 million fibers per liter. Asbestos or asbestos-like fibers may gain access to water supplies as a result of mining (Lake Superior), presence of natural serpentine or amphibole deposits in their water sheds (Seattle, Washington, San Francisco, California), or under certain conditions through the use of asbestos-cement pipe by municipal water supplies (EPA, 1980). In the latter instance erosion of fibers is associated with the "aggressiveness" of the water, a term representing a mathematical expression of pH, alkalinity, and calcium content. It was estimated that 68.5% of U.S. water systems utilize water that is potentially capable of eroding asbestos-cement pipe (EPA, 1980).

Furthermore, beers and wines could contain asbestos, possibly as a consequence of the use of asbestos filters in the preparation of these products (Cunningham and Pontefract, 1971). The ingestion of rice treated with asbestos containing talc has been hypothesized to be associated with an increased incidence of stomach cancer (Merliss, 1971a and 1971b).

A number of studies has provided evidence that ingestion of asbestos in either food or water can result in the migration of asbestos fibers through the gastrointestinal mucosa and distant organ sites in humans (Carter and Taylor, 1980), rats (Cunningham, et al., 1977), and baboons (Storeygard and Brown, 1977). Electron microscopic studies confirmed the presence of amphibole mineral fibers in the urine of people who ingested water containing these fibers (Cook and Olso, 1979).

Harrington, et al., (1978) failed to detect an association between the use of asbestos cement pipe for municipal water supplies and the incidence of gastrointestinal cancer. In a study of cancer incidence in the San Francisco Bay area, (Cooper, et al., 1978) reported a statistically significant trend for the incidence of several cancer types including stomach, gallbladder, esophageal and peritoneal cancer when analyzing census tracts on a gradient of low to high asbestos content in municipal water. In subsequent studies, Cooper, et al., (1979) confirmed the association between asbestos levels in San Francisco Bay area drinking water and cancer of the digestive tract.

Studies in animals have shown that the inhalation of asbestos can produce lung carcinoma and mesothelioma in the pleural cavity. Intrapleural, intratracheal and intraperitoneal injection of asbestos will also produce neoplasia in several species of laboratory animals. A review of the results of these animal studies is given by Levine (1978).

Asbestos (chrysotile, amosite and crocidolite) has been shown to be cytotoxic in vitro to human embryonic intestine and mouse epithelial-like colon derived cells and rat liver epithelial cells (Reiss et al., 1979). However, chrysotile asbestos was far more cytotoxic than the amphibole fibers and effects were more pronounced in the intestine derived cells than those from the liver. Asbestos was also found to be cytotoxic to Syrian hamster peritoneal macrophages (Bey and Harrington, 1971).

Reiss et al. (1979) showed that the above three forms of asbestos were not mutagenic using the HGPRT locus/resistance to 6-thioguanine assay system. In addition, no mutagenic activity was demonstrated using chrysotile, amosite or crocidolite asbestos in E. coli or S. typhimurium systems (Chamberlain and Tarmy, 1977). Therefore, it can be concluded that asbestos is not genotoxic but rather an epigenetic carcinogen of the solid state type (Weisburger and Williams, 1979).

In 1973 the National Institute of Environmental Health Sciences and the Environmental Protection Agency cosponsored a symposium on the possible biological effects of ingested asbestos (EHP, 1974). A concensus conclusion of that conference was that a paucity of data existed concerning the effects of ingested asbestos and that specific research was needed.

A Subcommittee of the DHEW Committee to Coordinate Toxicology and Related Programs subsequently reviewed existing data, and prepared a draft research protocol that the Committee felt was responsive to the major public health consensus. The protocol was widely distributed within and outside the government for comment. Based on the comments received, a revised final protocol was developed which required long-term animal toxicology studies whereby ingestion of several forms of asbestos would be evaluated for carcinogenic effect. The forms of asbestos to be studied included chrysotile (a serpentine asbestos), amosite and crocidolite (representative of amphibole asbestos), and a nonfibrous tremolite which contained low levels of asbestiform fibers.

All materials were to be tested in the Fischer 344 strain of rat whereas two forms of asbestos were to be tested in hamsters. All studies were to encompass the lifetime of the animal, defined as the period from which the animal commences eating solid food until death.

This report represents the results of those studies undertaken to determine the effects of chrysotile asbestos in the diet fed to Syrian golden hamsters.

## II. MATERIAL AND METHODS

### Test Materials

Asbestos is a general term applied to certain natural mineral silicates when they appear in a fibrous form. Chrysotile is the fibrous member of the serpentine mineral group, its chemical structure is $Mg_6Si_4O_{10}(OH)_8$. Two chrysotile test materials were selected for testing and are referred to as Short Range (SR) and Intermediate Range (IR) Chrysotile. A principle structural difference in the two materials is the presence, in IR Chrysotile, of fibers extending into relatively large sizes both with respect to fiber length and diameter.

The SR Chrysotile was purchased from the Union Carbide Corporation, Niagara Falls, New York, who referred to the material as COF-25. The chrysotile was mined from the New Idria serpentine mass located in southwestern San Benito and western Fresno counties of California.

The IR Chrysotile was purchased from the Johns Manville Company who referred to the material as Plastobest-20. The chrysotile was obtained from the Jeffrey Mine, Asbestos, Quebec, Canada.

Each material was packaged in new fiberboard drums in quantities of 25 (Short Range) or 50 pounds (Intermediate Range) and stored with other forms of asbestos in a special room in a warehouse at Research Triangle Park, North Carolina. Each drum received a color marking unique to the specific asbestos type.

The homogenicity of the samples and the physical and chemical properties of the materials were extensively characterized by the Bureau of Mines, U.S. Department of the Interior, (Supt. of Documents No. I 28.23:8452); and the Fine Particle Laboratories, Illinois Institute of Technology Research Institute, Chicago, Illinois (Special Report and Addendum on project L6085, contract N01-ES-5-3157). Copies of these reports are available upon request from the National Toxicology Program. Selected chemical and physical properties which define differences between the two chrysotile samples are given below.

Mineralogical Composition

Short Range Chrysotile was detected at a volume-percent abundance of greater than 96; minor amounts of calcite, brucite, talc, feldspar, quartz, and opaques.

Intermediate Range Chrysotile was detected at a volume-percent of greater than 96; minor amounts of platy serpentine, calcite, brucite, pyroxene, talc, magnetite and other opaques.

Fiber Characteristics

| | Short Range | Intermediate Range |
|---|---|---|
| Surface area ($m^2/g$) | 59.0 | 27.9 |
| Density ($g/cm^3$) | 2.577 ± .022 SD | 2.607 ± .016 SD |
| Measurement, Transmission Electron Microscopy | | |
| fiber count/gram | $.6081 \times 10^{13}$ | $.1291 \times 10^{12}$ |
| median length (μm) | 0.66 | 0.82 |
| range of length (μm) | 0.088 - 51.1 | 0.104 - 783 |
| median diameter (μm) | 0.059 | 0.089 |
| range of diameter (μm) | 0.019 - 1.57 | 0.019 - 11.5 |
| median fiber aspect ratio (l/d) | 11.1698 | 8.435 |

Frequency Distribution by Length (μm); Optical Microscopy

| | Short Range | Intermediate Range |
|---|---|---|
| 10 Percentile | 1.3 | 1.4 |
| 20 Percentile | 1.7 | 1.9 |
| 30 Percentile | 2.2 | 3.0 |
| 40 Percentile | 2.6 | 5.4 |
| 50 Percentile | 3.1 | 14.0 |
| 60 Percentile | 3.8 | 29.0 |
| 70 Percentile | 4.5 | 48.0 |
| 80 Percentile | 5.8 | 76.0 |
| 90 Percentile | 7.8 | 130.0 |

### Chemical-Instrumental Analyses (expressed as weight percent)

| | Short Range | Intermediate Range |
|---|---|---|
| $Al_2O_3$ | 0.66 | 1.47 |
| CaO | .32 | .05 |
| $Fe_2O_3$ | 2.02 | 2.93 |
| MgO | 40.62 | 40.62 |
| $K_2O$ | Not Detected | .08 |
| $SiO_2$ | 39.77 | 39.90 |
| $Na_2O$ | .01 | .04 |
| $TiO_2$ | .03 | .04 |
| MnO | .07 | .06 |
| $Cr_2O_3$ | .17 | .06 |
| NiO | .17 | .06 |
| $Co_2O_3$ | .01 | Not Detected |
| $CO_2$ | .78 | .51 |
| $H_2O$ | 1.54 | 1.17 |
| $H_2O^+$ | 12.69 | 12.81 |
| Benzene extracted organics | .026 | .011 |

### Test Diets

The diet used was NIH-31 open formula rodent diet prepared by Zeigler Brothers Inc., Gardners, PA. The test diet differed from the control diet through the incorporation to a level of 1% by weight of the appropriate chrysotile asbestos. Pilot studies determined that homogeneous mixing of asbestos in the diet occurred in a 55 cu. ft. Patterson Kelly "V" blender if the blender was loaded by alternate layering of feed and asbestos. All feed was pelleted using a Sprout-Waldron pelleter; the pellets were of oval configuration 3/8" x 3/4" size. Pelleted feed was packaged in 25 pound aliquots using standard paperfeed bags which were color coded to minimize the occurrence of feeding errors at the test laboratory.

Each lot of blended feed was analyzed for asbestos concentration; the results of these analyses are given in Appendix 1.

### Source and Specifications of Test Animals

Four groups (3 chrysotile and 1 amosite) of disease free, mated female outbred Syrian golden hamsters were obtained over a period of 20 weeks in 1977 from Charles River Lakeview Laboratories, New Field, NJ. The hamsters had been mated 6 days prior to shipping.

### Animal Maintenance (Table 1)

Upon arrival the mated female hamsters were weighed and sorted into weight ranges. They were then distributed randomly between control and treatment groups which were housed in separate rooms. The first shipment of mated females was assigned to the short range (SR) chrysotile study, the second to the intermediate range (IR) chrysotile study, the third shipment to the IR chrysotile plus DMH study, the fourth group to the amosite study and their respective control groups. Each dam was placed in an individual cage with filter top in their respective rooms. Treated or control food was provided *ad libitum* by placing on the floor of each cage. Water was provided *ad libitum* via water bottles. The hamsters were not handled except to change the cages just before the litters were due to be born. Once the litters were born they were left

Table 1. Materials and Methods for Animal Maintenance

| Item | Manufacturer | Specifications | Frequency of Change or Cleaning |
|---|---|---|---|
| Cages | Maryland Plastics<br>New York, NY | Econo-Cage<br>Polycarbonate<br>19" x 10 1/2" x 8" | IX/wk |
| Racks | Bussy Products | Stainless Steel<br>20 cages/rack | 1X/month |
| Bedding | Ab-sorb-Dri, Inc.<br>Rochelle Park, NJ | Hardwood<br>50 lb/bag | 1X/wk |
| Cage tops | Able Molded Plastics, Inc.<br>Chicago, IL | GE Lexan<br>Polycarbonate | 1X/month |
| Cage Filter | Associated Air Filter Co.<br>Rosemont, IL | 3" Diameter cut from FG50 Filter Mats | 1X/month |
| Metal Holder for Cage Filter | C.D. Cash Manufacturing<br>Chicago, IL | Outer shell with screen and baffle<br>Inner shell with screen | 1X/month |
| Snap Ring for Holder | Keats-Lorenz Spring Co.<br>Chicago, IL | Phosphor Bronz<br>Spring tempered | 1X/month |
| Feed dish with Metal Lips | W. Braun Company<br>Chicago, IL | 16 oz. Opal or clear glass jars | 1X/wk |
| Feed Follower | Unifab Corp.<br>Kalamazoo, MI | Stainless Steel with 7 holes | 1X/wk |
| Water Bottles | Continental Glass Co.<br>Chicago, IL | Pint Flint glass | 1X/wk |

Table 1 (Continued). Materials and Methods for Animal Maintenance

| Item | Manufacturer | Specifications | Frequency of Change or Cleaning |
|---|---|---|---|
| Watering Tube | Wahmann Mfg. Co.<br>Timonium, MD | Stainless Steel<br>5/16" OD 7 1/2" length with 120° bend 1 1/2" from bottle | 1X/wk |
| Feed | Zeigler Brothers<br>Gardners, PA | NIH 31 Diet<br>25 or 50 lb bags | 1X/wk |
| Cage & Bottle Washer | Blakeslee<br>Cicero, IL | Tunnel Wash | Daily/Monthly Check Maintenance |
| Autoclaves | American Sterilizer | Models<br>1) Medallion<br>2) RSP (Vacumatic S) | Bimonthly Maintenance |
| Washing Compounds | Economics Labs, Inc.<br>St. Paul, MN | Spearhead<br>Lime Away | |
| Room Air Filters (Exhaust) | (Roughing Filters)<br>Air Filter Equip. Corp.<br>Chicago, IL | Amer-Glass Filters<br>Type G filters | |
| | (Bag Housing)<br>Pure Air Filter<br>Chicago, IL | Dri Pak<br>2100 H Class II | |
| | | Absolute Filters<br>Am. Air Filter Astrocel | |
| Rack Washer | Metal Wash Machinery<br>Elizabeth, NJ | Mark V | Daily/Monthly Check Maintenance |

undisturbed until approximately 10 days of age. At this time the cages were changed weekly until the offspring were 4 weeks of age, at which time they were weaned.

At weaning the offspring were individually weighed and separated by sex. The test groups were randomly placed into groups of 3 males or 3 females which were housed in polycarbonate cages for the remainder of the lifetime study. The dams were killed at this time. Twenty male and 20 female offspring were removed from the study for endo- and ectoparasite examination (see Appendix B) to confirm that the test groups were of a desired health status. Extra hamsters were not discarded at this time in case of sexing errors. Approximately 6 weeks after weaning (10 weeks of age) all mis-sexed hamsters were killed along with their cage mates and were replaced with these alternates which had been treated in an identical manner. The remaining extra hamsters were killed. The experimental design insured that ingestion of asbestos spanned the entire phase of solid food consumption during the lifetime of the animal. Food consumption was not determined because of the hamsters habit of sequestering its feed in the bedding. Control hamsters were housed in separate rooms. The number of animals in the study is shown in Table 2.

Starting at 6 weeks of age male and female hamsters in the IR chrysotile/1,2-dimethylhydrazine dihydrochloride (DMH) study (Table 2) were given oral doses of DMH (4 mg/kg) every other week for a total of 5 doses. The dose of DMH used in this study was based on the results of a pilot study carried out previously in the same facility (see Pilot Study section for details). The DMH (Aldrich Chemical Co., Milwaukee, WI) was used as received and was dissolved in 0.9% saline to a concentration of 1.5% (15 mg/ml). This stock solution was then diluted with saline to give the appropriate concentration for dosing. The solutions were made up within one hour prior to dosing the hamsters. All dosing was completed in less than 3 hours. The DMH was analyzed after each dosing (Appendix C).

During the test period the temperature was maintained at 22°C ± 2° and the relative humidity ranged from 40-80%. To minimize contamination of room air with asbestos, each cage was totally enclosed. Incoming air to the cages was filtered through fiberglass filters while exit air was filtered through a fiberglass roughing filter followed by a bag housing filter. The cage atmosphere was negative to the room and the room was maintained at a slightly negative atmosphere in relation to corridor air. Air flow within the animal rooms was maintained with a minimum of 20 air changes per hour. Fluorescent lighting was provided on a 12 hour per day cycle.

### Clinical Examination and Pathology

All hamsters were observed daily for signs of toxicity. Individual animal body weights were recorded weekly for the duration of the study. All animals were allowed to die or were killed in a moribund condition with pentobarbitol sodium. A complete post mortem examination was performed on all animals unless severely cannibalized or autolyzed. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group (Table 2).

Since the gastrointestinal tract was chosen as one of the target organs prior to the study, it was handled in a slightly different manner than what is normally conducted in standard rodent life time bioassays. The entire esophagus was opened and pinned with the exterior surface adjacent to cardboard prior to placement in fixative. The stomach and cecum were prepared in a similar manner.

Table 2. Disposition of Hamsters from the Chrysotile Asbestos Feeding Study

| Group | Sex | On Test | Histopathologic Evaluation | Missing | Cannibalized | Autolyzed | Missexed |
|---|---|---|---|---|---|---|---|
| SR Chrysotile Control | M | 126 | 115 | 0 | 3 | 6 | 2 |
| | F | 126 | 114 | 0 | 1 | 6 | 5 |
| IR Chrysotile Control | M | 126 | 116 | 0 | 0 | 8 | 2 |
| | F | 126 | 119 | 0 | 0 | 4 | 3 |
| DMH and IR Chrysotile Control | M | 125 | 119 | 0 | 0 | 3 | 3 |
| | F | 128 | 120 | 1 | 0 | 2 | 5 |
| Amosite Control | M | 127 | 122 | 0 | 0 | 4 | 1 |
| | F | 126 | 119 | 1 | 0 | 1 | 5 |
| SR Chrysotile | M | 253 | 233 | 0 | 1 | 10 | 9 |
| | F | 252 | 228 | 1 | 0 | 17 | 6 |
| IR Chrysotile | M | 251 | 245 | 0 | 0 | 3 | 3 |
| | F | 252 | 244 | 1 | 0 | 3 | 4 |
| DMH | M | 127 | 127 | 0 | 0 | 0 | 0 |
| | F | 126 | 122 | 0 | 0 | 1 | 3 |
| DMH and IR Chrysotile | M | 176 | 173 | 0 | 0 | 2 | 1 |
| | F | 174 | 161 | 3 | 0 | 6 | 4 |

Two cm lengths of duodenum, ileum and 2 portions of jejunum were placed unopened in fixative so that cross sections of these tissues were available for histopathologic examination. The remaining small intestine was opened and gently washed with saline after which it was carefully examined. Suspect lesions were processed separately and identified individually as to location. The entire colon with anus was opened, examined and pinned similarly to cardboard prior to fixation. The size and location of masses were recorded. Masses greater than 1 mm diameter were removed as separate specimens for processing. After fixation the colon was "carpet-rolled" starting at the posterior end with the mucosal surface inward prior to embedding.

All tissues were fixed in 10% neutral buffered formalin, sectioned, and stained with hematoxylin and eosin. Tissues/organs examined microscopically were: tissue masses, the above mentioned portions of gastrointestinal tract, mesenteric and bronchial lymph nodes, mammary gland, salivary gland, thigh muscle, bone marrow (sternum), nasal cavity with turbinates, larynx, trachea, lungs and bronchi, heart, thyroid, parathyroid, liver, gall bladder, pancreas, spleen, kidneys, adrenal glands, urinary bladder, seminal vesicles/prostate/ testes, ovaries/uterus, brain, pituitary gland, eyes and spinal cord.

The pathologic findings of the contractor pathologist were subjected to a quality assurance review by an independent pathology contractor. This review consisted of an examination of all tumors diagnosed by the original pathologist, target organs (gastrointestinal tract) from all animals, all organs from 10% randomly selected hamsters and a tissue count on all animals. Sections from all tumors and any other organ where a discrepancy existed between original and reviewing pathologist were submitted to the NTP Pathology Working Group (NTP/PWG) for subsequent review. All slides where there was a discrepancy in tumor diagnosis between the original pathologist and the NTP/PWG were returned to the original pathologist for re-evaluation. The tables shown in this report represent the original pathologists final diagnosis. Reported separately are cases where the original pathologist did not agree with the NTP/PWG.

## Data Recording and Statistical Methods

The individual animal pathology data on this experiment were recorded in the Carcinogenesis Bioassay Data System. The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, and individual pathologic results.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Differences in survival were evaluated by Cox's (1972) life table method.

As noted earlier, concurrent studies were conducted in this laboratory with another form of asbestos (amosite) using exactly the same protocol (NTP, 1981). Although the results of these studies are not given in this report, the amosite controls were included with the chrysotile control groups for statistical purposes as part of the pooled controls.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

For the statistical analysis of tumor incidence data, two different methods of adjusting for intercurrent mortality were employed. Each used the classical methods for combining contingency tables developed by Mantel and Haenszel (1959).

The first method of analysis assumed that all tumors of a given type were fatal, i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the treated and control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results were then combined by Mantel-Haenszel methods to obtain an overall probability (P) value. This method of adjusting for intercurrent mortality is Cox's life table method (1972).

The second method of analysis assumed that all tumors of a given type were "incidental", i.e., they were merely observed at autopsy in animals dying of an unrelated cause. According to this approach, the proportions of animals found to have tumors in treated and control groups were compared in each of five time intervals. For male hamsters these time intervals were 0-52 weeks, 53-78 weeks, 79-92 weeks, 93-103 weeks, and greater than 103 weeks. For female hamsters whose median survival was considerably less than the males, the time intervals were 0-44 weeks, 45-52 weeks, 53-60 weeks, 61-68 weeks and greater than 68 weeks. The denominators of these proportions were the number of animals actually autopsied during the time interval. The individual time interval comparisons were then combined by the previously described methods to obtain a single overall result. (See Peto, et al., 1980).

In addition to these tests, one other set of statistical analyses was carried out for each primary tumor: the Fisher exact test based on the overall proportion of tumor-bearing animals (Gart, et al., 1979). All reported P values are one sided. Except where noted, the results of the three alternative analyses gave similar results.

## Pilot Study for Dose Setting of Intestinal Carcinogen

This pilot study was designed to determine the dose of a known intestinal carcinogen that would produce a low incidence (10 ± 5%) of intestinal cancer and relatively little toxicity or neoplasia at other sites in the body. It was conducted in a similar manner to that reported in rats (McConnell, *et al.*, 1980). The chemicals chosen were methylazoxymethanol acetate (MAM) and 1,2-dimethylhydrazine dihydrochloride (DMH) (Aldrich Chemical Co., Milwaukee, WI). The chemical which most nearly met these two criteria would be used in the cocarcinogen study.

MAM or DMH was used as received and dissolved in 0.9% saline to a concentration of 1.5% (15 mg/ml) which was then diluted with saline to give the appropriate concentration for dosing. The solutions were made up within one hour prior to dosing the animals. All dosing was completed in less than 3 hours following preparation of the solutions in order to prevent possible decomposition.

Personnel wore full protective clothing and activated charcoal respirators while in the rooms during the actual dosing and for an additional 2 weeks following the last dose. After this time normal safety precautions were used.

Four week old male and female Golden Syrian hamsters were obtained (A.R. Schmidt Co., Madison, WI) for this pilot study. They were acclimatized to their environment 2 weeks during which 2 males and 2 females were randomly chosen for qualitative disease diagnosis as described before. Caging, bedding, and feeding were carried out as described before (Table 1). At 6 weeks of age the hamsters were sorted by weight and assigned randomly to the treated groups prior to compound administration. DMH or MAM solutions were administered by gastric intubation, 0.2 ml/kg body weight every other week for 10 weeks (5 doses). Dose levels and group sizes are shown in Table 3. The group sizes were selected to include the largest numbers of animals in dosage groups where it was speculated that the desired affects would be observed. The study was terminated 9 months following the last dose of the carcinogen.

All hamsters were observed daily and were weighed once/week, although statistical analyses were not performed on body weight gain. Clinical signs were not recorded unless considered pertinent to pathological observations. Fecal samples were analyzed for the presence of occult blood (clinitest tablets) 3 months following the dosing regimen (blood in the feces is often associated with the presence of intestinal neoplasms). A slight positive reaction is normally observed due to the presence of undigested myoglobin in the feed. Any increase in intensity was considered to be a qualitative indication of occult blood.

All hamsters which died or which were killed in a moribund condition and those killed at the end of the study were subjected to a complete post mortem examination as described before. While all hamsters were necropsied, not all animals were submitted for histopathological evaluation. The tissues from animals selected for histology were taken predominantly from the control groups of hamsters, animals showing macroscopic tumors and those hamsters treated with lower doses of DMH, since it was obvious early in the study that DMH produced less hepatic toxicity than MAM (see below). Tissues routinely selected for histopathological examination included representative portions of the entire gastrointestinal tract, liver, kidneys, mesenteric and colo-rectal lymph nodes and any macroscopically visable or suspect lesions. Methods used for the handling of these tissues were identical to that described previously.

Only the highest dose (30 mg/kg) of both MAM and DMH caused a marked affect on body weight gain. Females were more effected than males. The 30 mg/kg and 15 mg/kg doses of both MAM and DMH markedly decreased the survival rate with females again being more affected than males at the same dose. Many of these hamsters died early in the study due to hepatic toxicity.

The majority of the animals which received the two higher doses (30 and 15 mg/kg) of both compounds which did not die early in the study due to toxic hepatitis (see below) had macroscopically visable masses in the colon and/or cecum; the colonic masses were often adhered to the abdominal wall and, in many instances, contained large abscesses. However because of initial animal loss due to hepatic toxicity tumor, an evaluation of tumor/dose incidence in these groups was not possible.

The tumors of the colon were morphologically comparable to those described in a similar study in Fischer 344 rats using DMH (McConnell, et al., 1980) and in a single dose study of MAM (Ward, 1975). These neoplasms varied from adenomatous polyps to tubular or mucinous adenocarcinoma with invasion through the muscle wall and local metastases to regional lymph nodes. Cystic epithelial hyperplasia of the cecum was commonly observed but was probably not related to carcinogen exposure.

In addition to effects on the gastrointestinal tract, marked hepatic toxicity (dose related) was observed both at necropsy and histopathologically, and more so in the hamsters exposed to MAM. Affected livers had a diffuse nodular appearance with a coarse granular surface. Some livers contained large sac-like structures filled with blood which at times replaced most of the affected lobe. Microscopically, the liver lesions encompasses a variety of changes which included focal necrosis with blood filled spaces, hepatocellular vacuolization, cellular and nuclear pleomorphism, and focal nodular hyperplasia. Hepatoxtoxicity in DMH treated hamsters was judged to be minimal at doses at 7.5 and 4 mg/kg and was not observed below this dose. Nonchemical related lesions in the liver included periportal amyloidosis, bile duct proliferation and intrahepatic biliary cysts. These changes were found to a greater or lesser degree in almost every hamster.

In those hamsters which survived the early hepatic toxicity, there was an increasing mortality apparently due to severe nephropathy. This appeared in all dose groups and the controls. The kidneys from these animals were pale, granular, and had a thin cortex. These animals were usually emaciated and often had a granular appearance of the liver. Microscopically, the kidneys were characterized by diffuse and often massive accumulations of amyloid. The glomerulus seemed to be primarily affected but involvement of the entire cortex and to a lesser extent the medulla was rapid. An inflammatory component was conspicuously absent.

In conclusion both chemicals caused a dose related induction of intestinal tumors but DMH had fewer toxic side effects than MAM and for this reason was chosen for the cocarcinogen study. The dose chosen for females was relatively straight forward; 4 mg/kg produced the desired incidence (11%) of tumors of the large intestine (benign and malignant) while 7.5 mg/kg caused a high incidence (50%) of intestinal tumors and 1 mg/kg did not cause any malignant tumors (Tables 4 and 5). The data for the males was more difficult to interpret because of a lack of a clear dose response. Again 4 mg/kg was chosen for the cocarcinogen study because 7.5 mg/kg caused too high an incidence of malignant tumors. Even though malignant tumors were not observed at the 4 mg/kg dose, they were observed at a low incidence in the 1 mg/kg dose group which would suggest that 4 mg/kg should produce a higher yield of intestinal tumors in the subsequent study.

Table 3. Dose Levels and Group Sizes of Hamsters Used in the Dose-Response Studies of Methylazoxymethanol (MAM) and 1,2-Dimethylhydrazine (DMH)

| Dose Level (mg/kg b.w.) | Group Size | | |
|---|---|---|---|
| | Males | Females | Total |
| | MAM | | |
| 30 | 18 | 18 | 36 |
| 15 | 21 | 21 | 42 |
| 7.5 | 27 | 27 | 54 |
| 4 | 27 | 27 | 54 |
| 1 | 27 | 27 | 54 |
| 0.2 | 30 | 30 | 60 |
| 0 (0.9% saline control) | 30 | 30 | 60 |
| | DMH | | |
| 30 | 18 | 18 | 36 |
| 15 | 21 | 21 | 42 |
| 7.5 | 27 | 27 | 54 |
| 4 | 27 | 27 | 54 |
| 1 | 27 | 27 | 54 |
| 0.2 | 30 | 30 | 60 |
| 0 (0.9% saline control) | 30 | 30 | 60 |

Table 4. Intestinal Tumor Incidence in Hamsters Treated with 1,2-Dimethylhydrazine

| Group | Effective No. of Animals* | No. of Animals for Histopath. | TBA** | % TBA*** | Total No. of Tumors | |
|---|---|---|---|---|---|---|
| | | | | | Benign | Malignant |
| I. Males | | | | | | |
| Saline (DMH) | 29 | 9 | 2 | 7% | 2 | 0 |
| DMH, 7.5 mg/kg | 27 | 10 | 7 | 26% | 14 | 25 |
| DMH, 4 mg/kg | 27 | 7 | 4 | 15% | 5 | 0 |
| DMH, 1 mg/kg | 27 | 14 | 6 | 22% | 18 | 2 |
| DMH, 0.2 mg/kg | 27 | 14 | 6 | 22% | 9 | 0 |
| II. Females | | | | | | |
| Saline (DMH) | 29 | 18 | 2 | 7% | 2 | 0 |
| DMH, 7.5 mg/kg | 26 | 13 | 13 | 50% | 29 | 115 |
| DMH, 4 mg/kg | 27 | 11 | 3 | 11% | 2 | 5 |
| DMH, 1 mg/kg | 27 | 13 | 3 | 11% | 4 | 0 |
| DMH, 0.2 mg/kg | 27 | 11 | 3 | 11% | 3 | 0 |

*Effective No. of Animals = original no. in group minus animals lost to autolysis.

**Based on histopathological evaluation = intestinal tumor bearing animals.

***$\% \text{ TBA} = \frac{\text{TBA}}{\text{Effective No. Animals}} \times 100$

Table 5. Total Number and Types of Intestinal Tract Tumors in Hamsters Treated with 1,2-Dimethylhydrazine

| Group | No. Animals Examined Histopath | Tumor Bearing Animals | Number of Tumors<br>Adenomatous Polyp | Adenoma, Sessile | Adeno CA (sessile) | Adeno CA (in situ) | Mucinous Adeno CA | Location |
|---|---|---|---|---|---|---|---|---|
| I. Males | | | | | | | | |
| Saline (DMH) | 9 | 2 | 2 | | | | | Colon |
| DMH, 7.5 mg/kg | 10 | 7 | 14 | | 13 | 12 | | Colon, Cecum |
| DMH, 4 mg/kg | 7 | 4 | 4 | 1 | | | | Colon, Cecum |
| DMH, 1 mg/kg | 14 | 6 | 11 | 7 | | 1 | 1 | Colon, Cecum |
| DMH, 0.2 mg/kg | 14 | 6 | 9 | | | | | Colon, Cecum |
| II. Females | | | | | | | | |
| Saline (DMH) | 18 | 2 | 2 | | | | | Colon |
| DMH, 7.5 mg/kg | 13 | 13 | 29 | | 28 | 86 | | Colon, Cecum |
| DMH, 4 mg/kg | 11 | 3 | 2 | | | 5 | | Colon |
| DMH, 1 mg/kg | 13 | 3 | 1 | 3 | | | | Colon |
| DMH, 0.2 mg/kg | 11 | 3 | 3 | | | | | Colon |

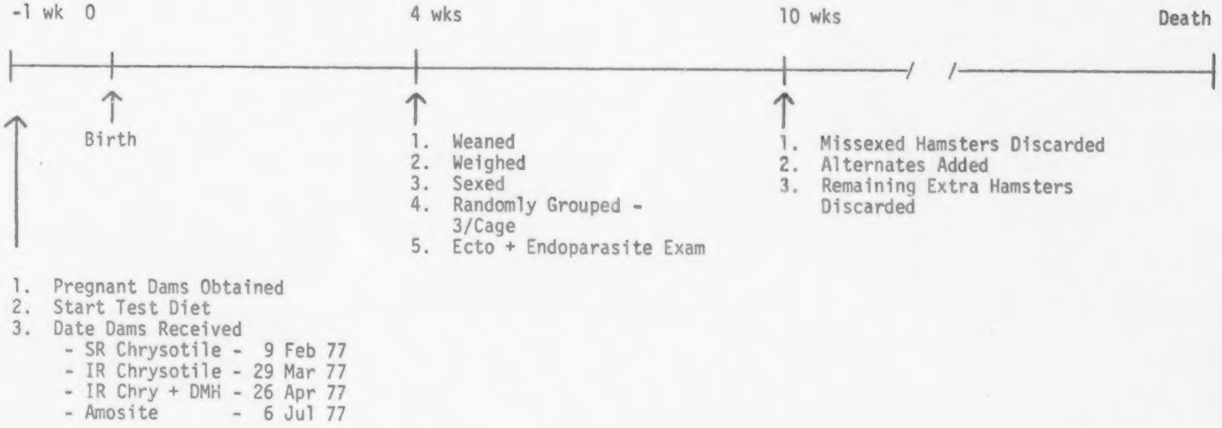
## III RESULTS

### Establishment of Test Groups

The experiment was designed to evaluate the effects of orally ingested chrysotile asbestos during the entire life of the animal starting from the time it was able to eat solid food. For this reason the mated female hamsters had been on the test diets for approximately 2 weeks when the first litters were born. Ten-15% of the females were either not pregnant, aborted, or their litters died immediately after birth. Several more dams died after showing a prolapsed rectum in the week following birth. The incidences of infertility and neonatal deaths were unrelated to the test diet. The litters were not manipulated or handled during lactation to minimize the chance that the mothers would reject or cannibalize their litters. Many of the pups which died during the nursing period were cannibalized by their mothers. In those pups in which a postmortem examination was possible, the stomachs were typically without food (milk) suggesting maternal rejection, or inability to compete with litter mates. None of these observations were compound related.

Approximately 2% of the offspring in all groups died between weaning and 14 weeks of age due to cage fighting or an enteritis of undetermined origin. Histologically the disease was compatable with the acute form of proliferative ileitis ("wet tail"), a common disease of hamsters. Combinations of cage fighting and enteritis were also observed. These deaths were not compound related, although cage fighting was more severe in the SR chrysotile and its temporal control group than in the other two portions of the study. Replacement hamsters were incorporated into the groups (in additional cages) to maintain group sizes until the animals were 10 weeks of age after which time no additional hamsters were added to the experimental groups. The extra hamsters were killed (Figure 1).

Figure 1. Schedule of Major Events in the Chrysotile Asbestos Study


-1 wk
0
4 wks
10 wks
Death
Birth
1. Pregnant Dams Obtained
2. Start Test Diet
3. Date Dams Received
- SR Chrysotile - 9 Feb 77
- IR Chrysotile - 29 Mar 77
- IR Chry + DMH - 26 Apr 77
- Amosite - 6 Jul 77
1. Weaned
2. Weighed
3. Sexed
4. Randomly Grouped - 3/Cage
5. Ecto + Endoparasite Exam
1. Missexed Hamsters Discarded
2. Alternates Added
3. Remaining Extra Hamsters Discarded

## Body Weight and Clinical Signs

Body weight gain was not adversely affected by any of the test diets including the group given 1,2-dimethylhydrazine (DMH) (Figures 2-7). In fact, chrysotile asbestos appeared to increase body weight in most of the treated groups. The inordinately sharp rise in weight gain of male and female hamsters in the SR Chrysotile study at week 29 and sharp decrease in male and female IR Chrysotile exposed animals at week 18 could not be explained but was felt to be a spurious observation. No compound related clinical signs were observed during the entire study. Occasional skin lesions and bite wounds were observed in both sexes but were more apparent in males, and became less of a problem after the hamsters were 20 weeks of age.

## Survival

Survival was not adversely affected by any of the test diets with the possible exception of DMH treated female hamsters (Figures 8-13). Survival rates were actually better in the SR and IR chrysotile groups relative to the temporal controls. The median life span of females (control and treated) was shorter than corresponding groups of males (Table 6). The median survival of control female groups was 57-61 weeks compared to 77-83 weeks for control male hamsters (Figures 8-13 and Table 6).

## Pathology

The number of hamsters available for histopathologic examination is shown in Table 2. The majority of animals not included in the pathologic analysis were excluded because of autolysis or cannibalism. Review of the clinical records of hamsters lost to autolysis or cannabalization gave no indication that such animals had neoplasia.

A variety of neoplasms was observed in control (Tables 7 and 8) and chrysotile exposed hamsters (Tables 9-14). The proportion of control male or female hamsters bearing primary tumors were not statistically different among the 4 control groups. Thus, statistical comparisons were made with pooled controls as well as temporal controls. Overall the male hamsters had a slightly higher rate of neoplasia than the females.

Those organs with greater than 4% incidence of neoplasia in treated or control groups were the adrenal gland, pancreas (Islets of Langerhans), parathyroid and reticuloendothelial system.

The only organ showing an increased rate of neoplasia in chrysotile treated hamsters compared to the controls was the adrenal cortex. In male hamsters the incidence of cortical adenomas was significantly increased ($p<0.05$) in the SR and IR chrysotile groups compared to the pooled controls but not in the DMH chrysotile group (Tables 9, 11 and 13). None of the chrysotile groups showed a sig~~nificant (~~$p<0.05$) increase in cortical adenomas relative to its temporal control groups. A similar increase in cortical adenomas was observed in the female IR chrysotile group compared to pooled controls but this also ceased to be significant when compared to the temporal control group.

Males and females treated with DMH did not show a significant ($p<0.05$) increase in intestinal neoplasia nor did the intermediate range chrysotile produce a higher rate of intestinal neoplasia in DMH treated animals (Table 15). A summary of all gastrointestinal tumors observed in this study is given in Table 15.

There were only two other instances where specific tumor types showed significant effects relative to pooled or temporal controls. Female hamsters treated with SR Chrysotile showed a significantly ($P<0.05$) decreased incidence of islet cell adenoma relative to pooled controls (Table 10). Male hamsters treated with DMH showed a significantly ($P<0.05$) increased incidence of leukemia or malignant lymphoma relative to pooled controls (Table 13).

Figure 2. Growth Curve of Male Hamsters Administered Short Range Chrysotile Asbestos in the Diet

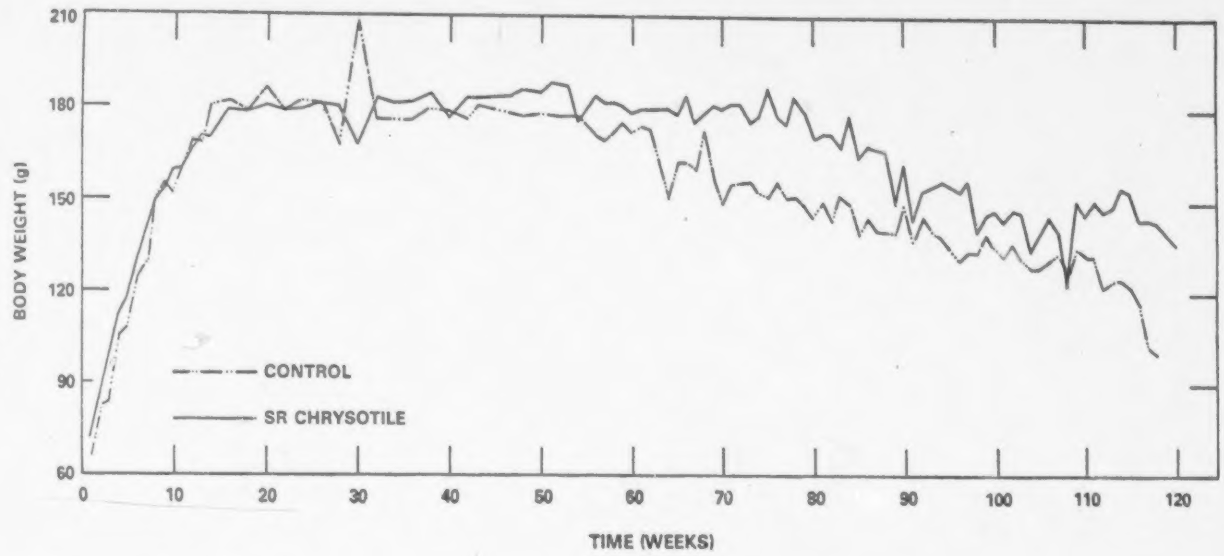


Figure 3. Growth Curve of Female Hamsters Administered Short Range Chrysotile Asbestos in the Diet

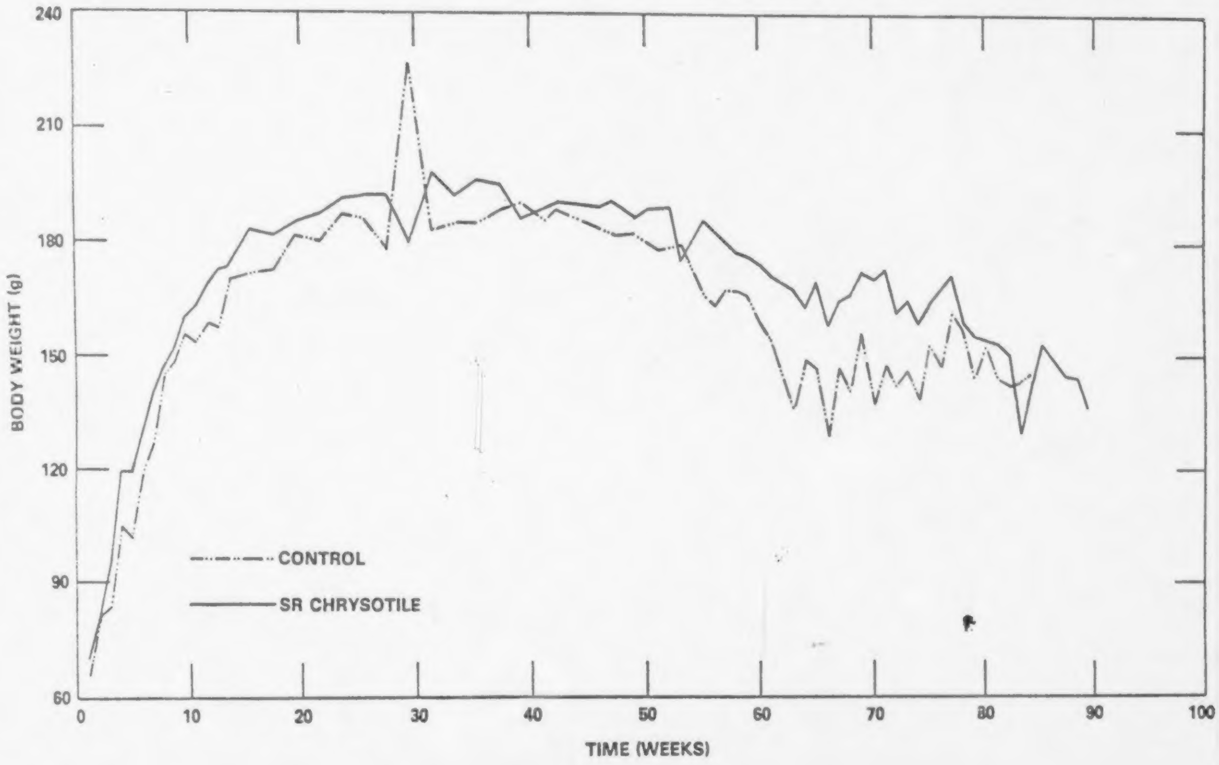


Figure 4. Growth Curve of Male Hamsters Administered Intermediate Range Chrysotile Asbestos in the Diet

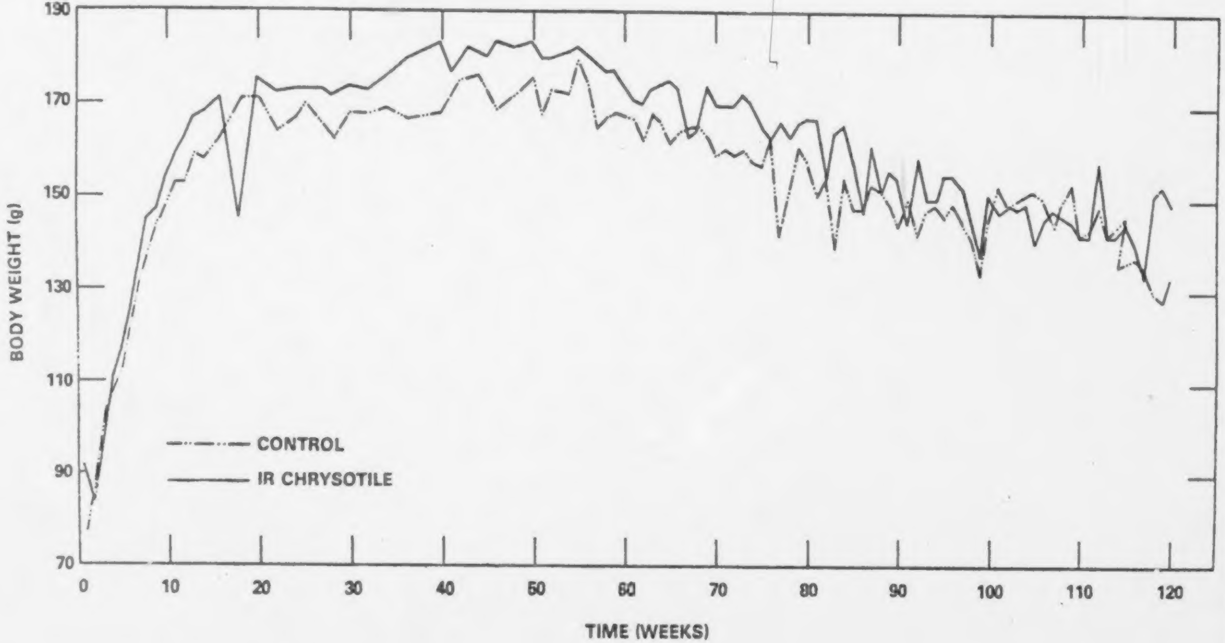


Figure 5. Growth Curve of Female Hamsters Administered Intermediate Range Chrysotile Asbestos in the Diet

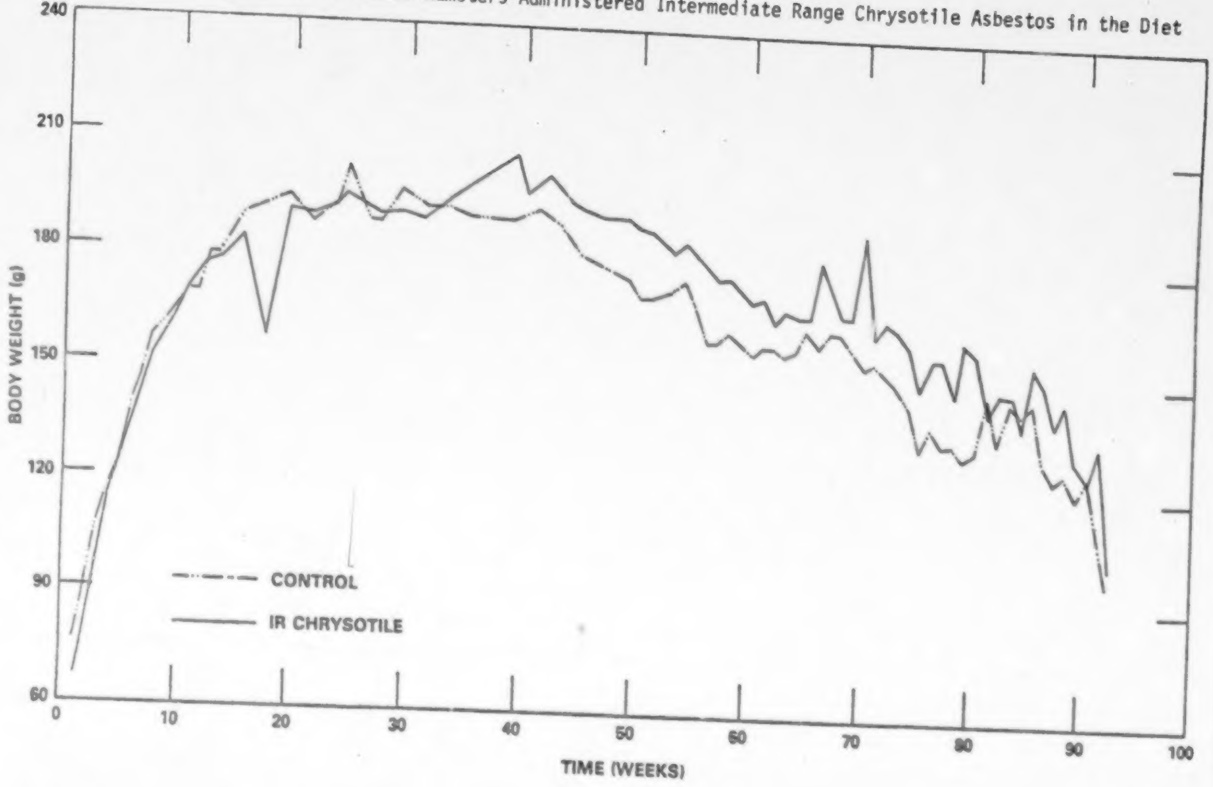


Figure 6. Growth Curve of Male Hamsters Administered 1,2-Dimethylhydrazine (DMH) by Gavage or DMH plus Intermediate Range Chrysotile Asbestos in the Diet

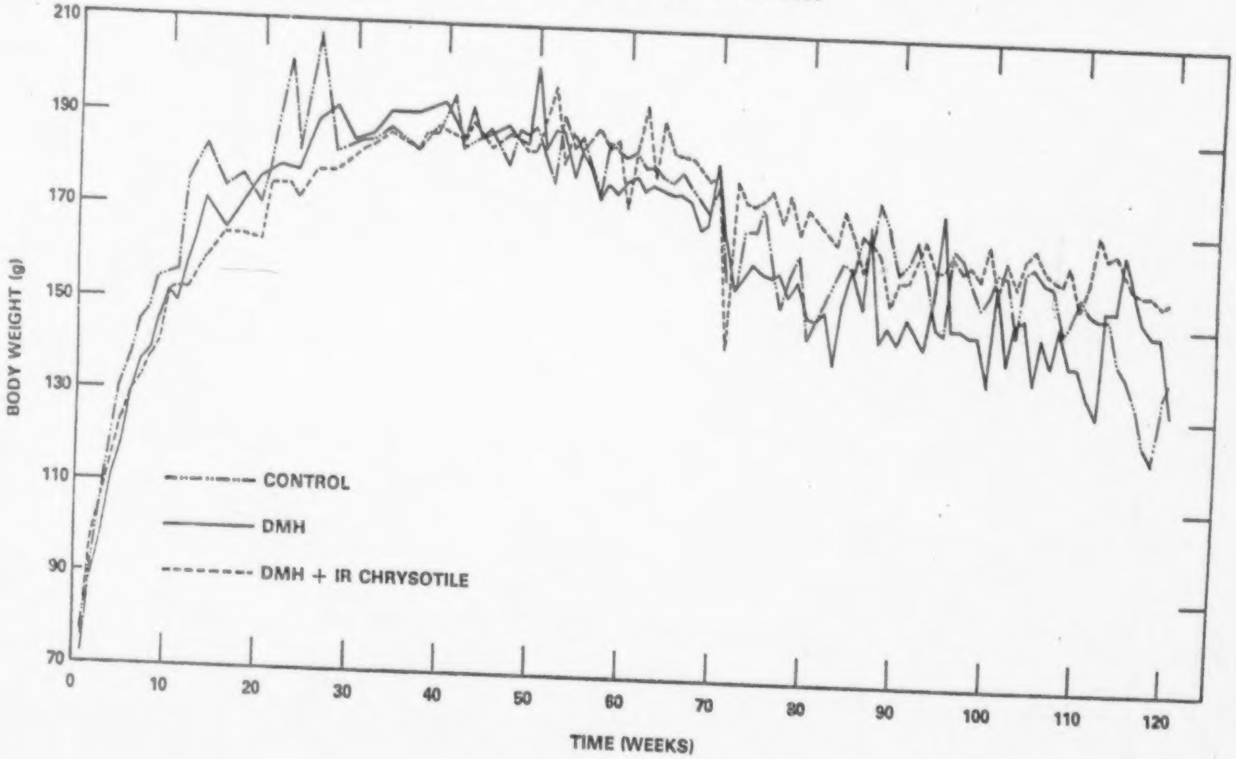


Figure 7. Growth Curve of Female Hamsters Administered 1,2-Dimethylhydrazine (DMH) by Gavage or DMH plus Intermediate Range Chrysotile Asbestos in the Diet

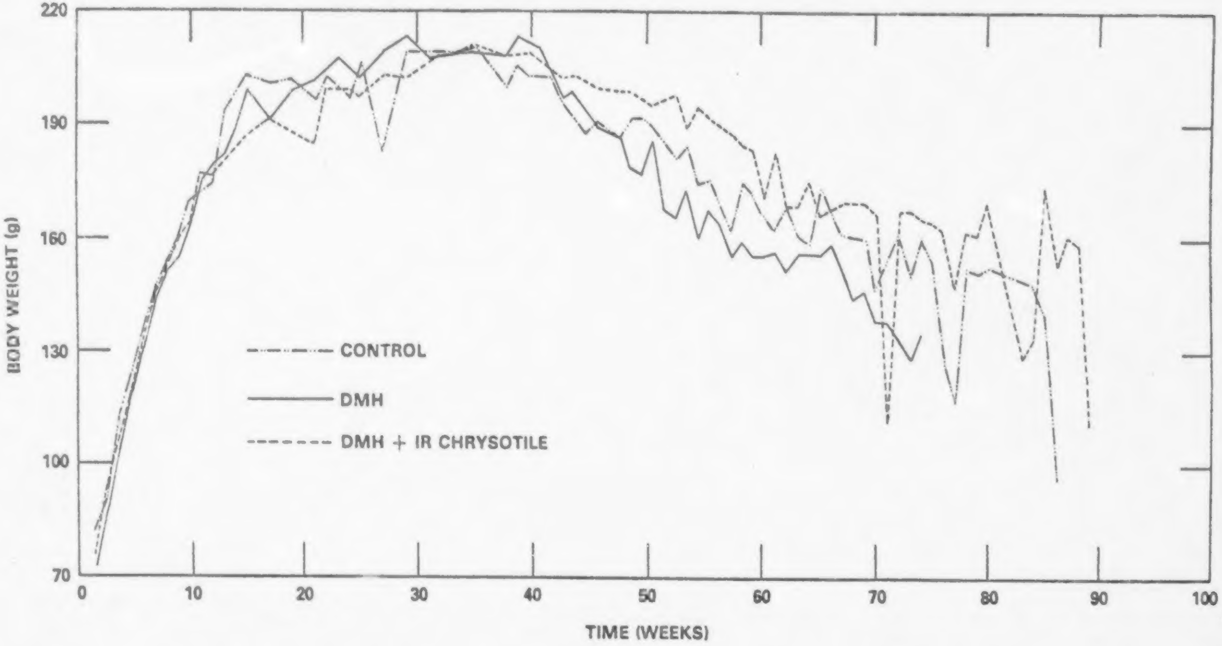
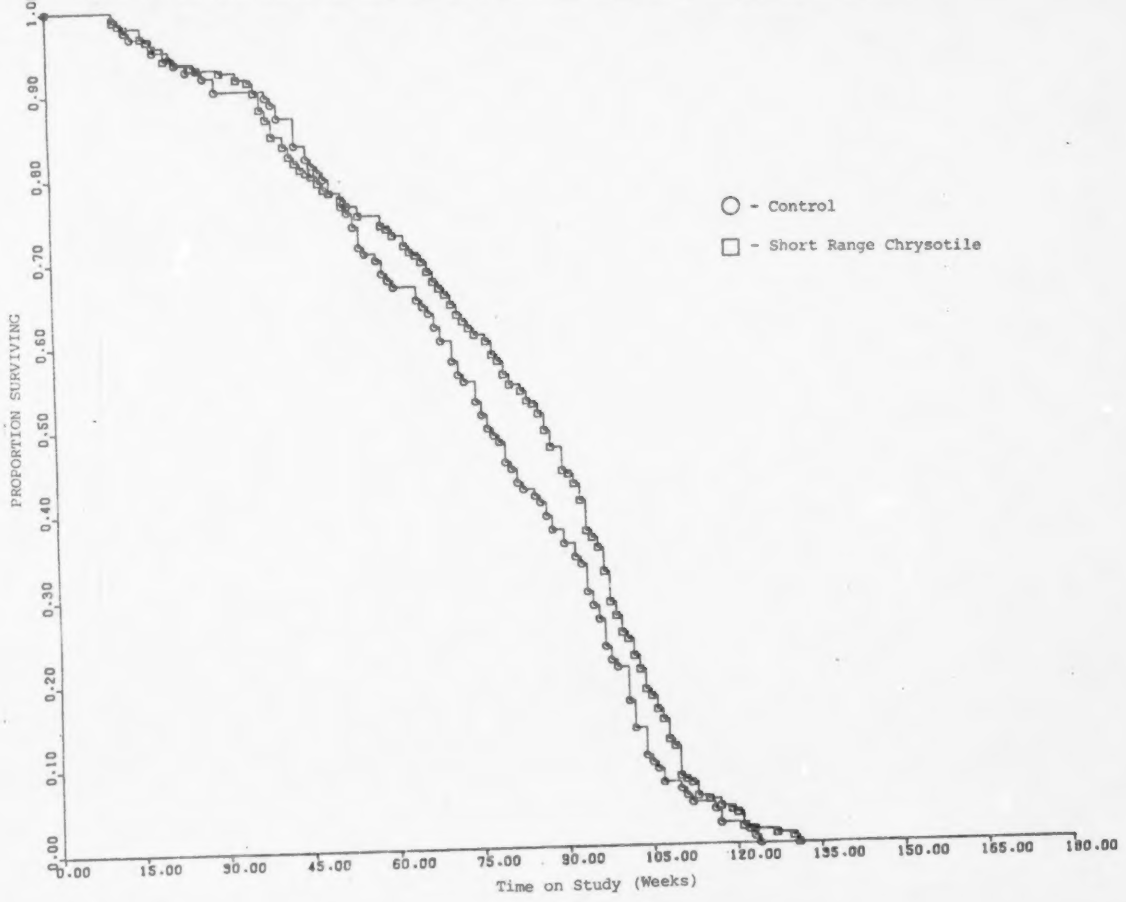


Figure 8. Survival Curve for Male Hamsters Receiving Short Range Chrysotile Asbestos.



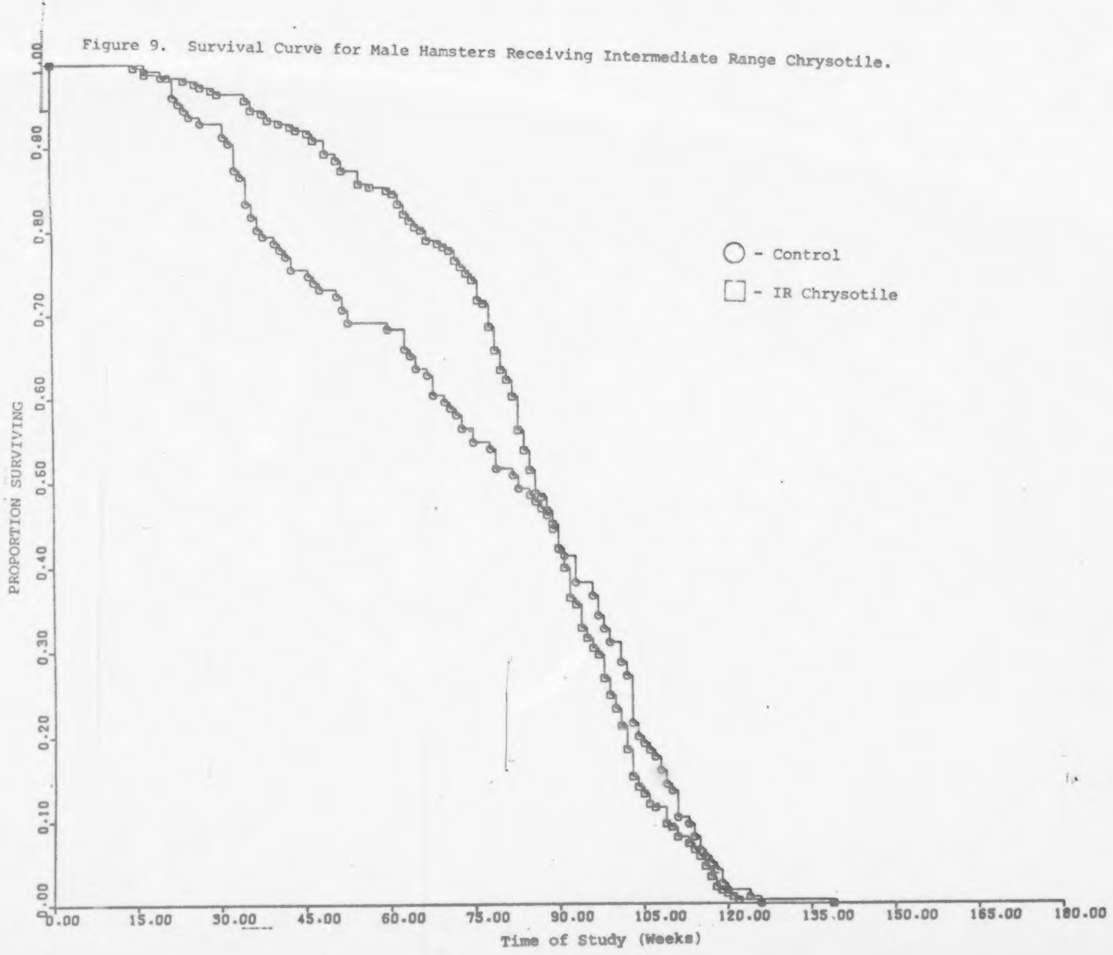
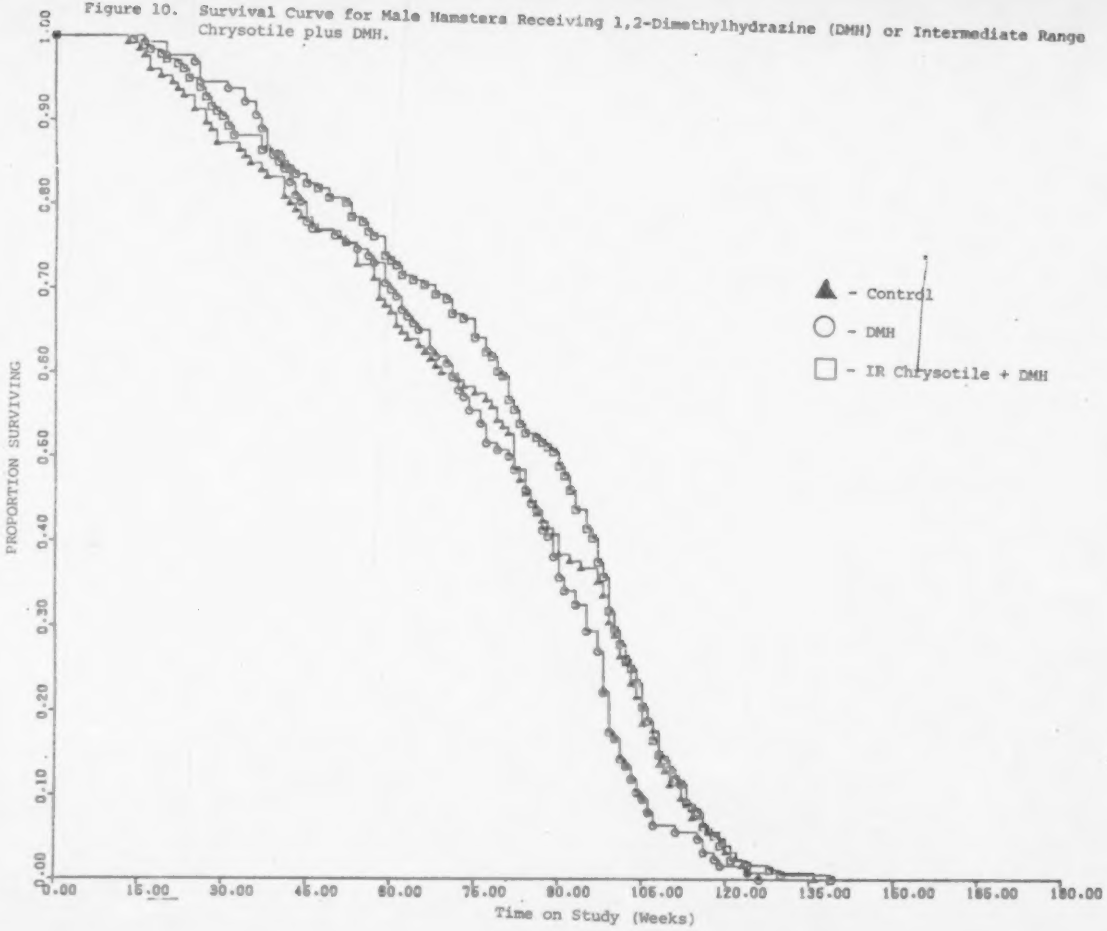


Figure 9. Survival Curve for Male Hamsters Receiving Intermediate Range Chrysotile.

Figure 10. Survival Curve for Male Hamsters Receiving 1,2-Dimethylhydrazine (DMH) or Intermediate Range Chrysotile plus DMH.



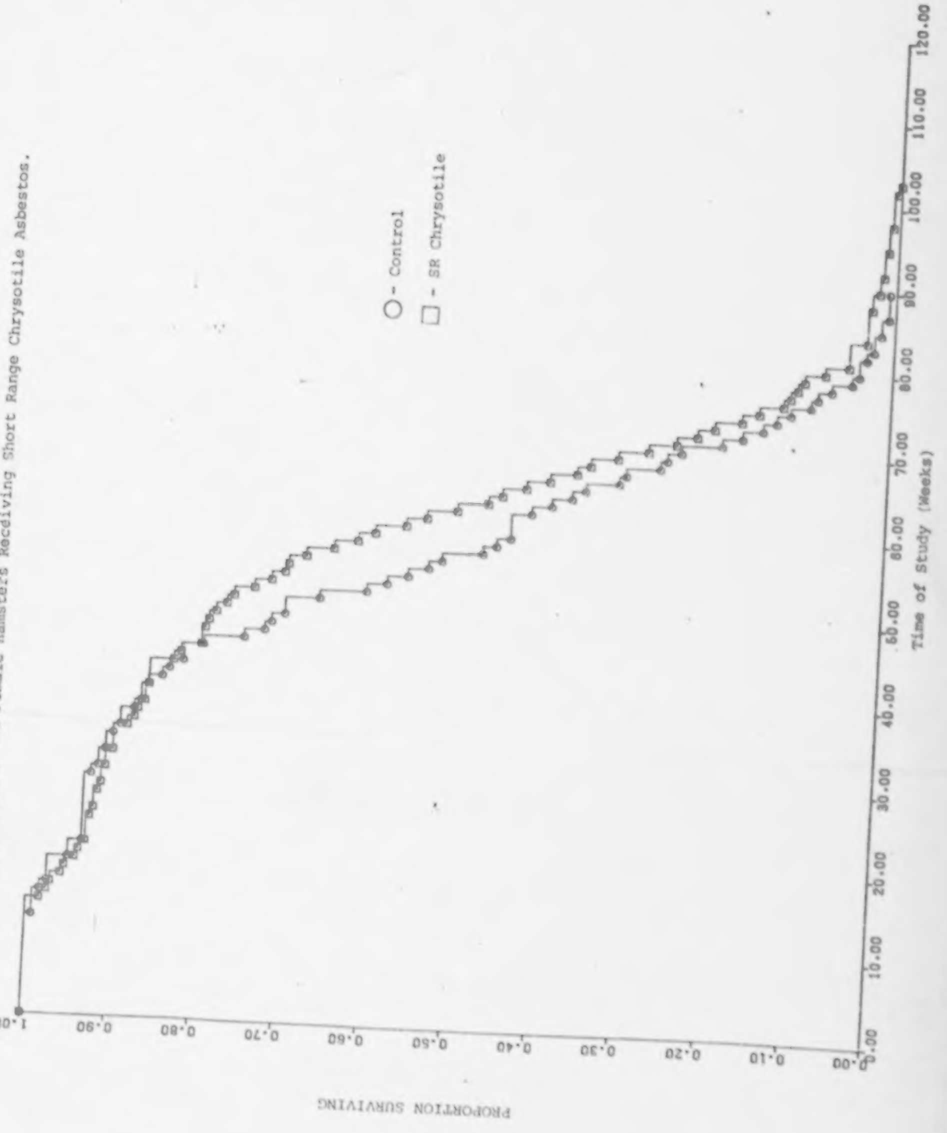


Figure 11. Survival Curve for Female Hamsters Receiving Short Range Chrysotile Asbestos.

Figure 12. Survival Curve for Female Hamsters Receiving Intermediate Range Chrysotile Asbestos.

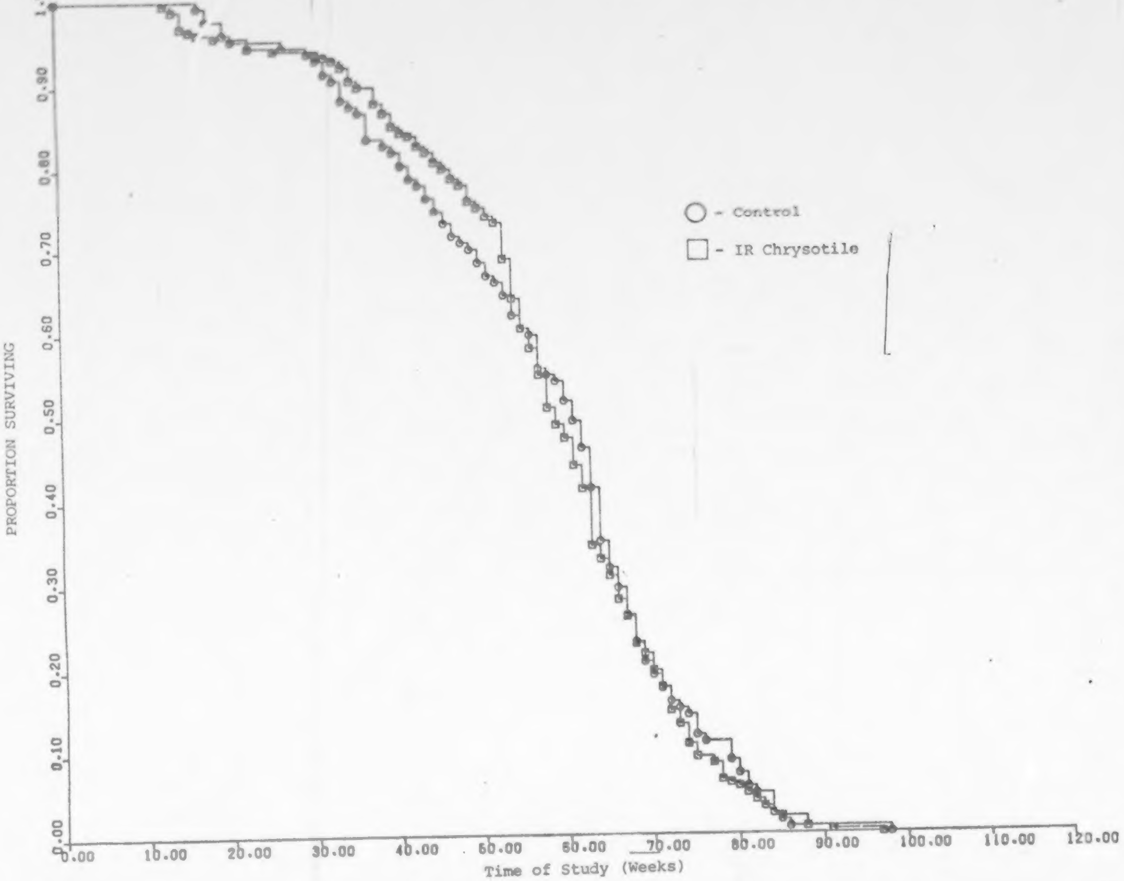


Figure 13. Survival for Hamsters Receiving 1,2-Dimethylhydrazine (DMH) or Intermediate Range Chrysotile Asbestos plus DMH.

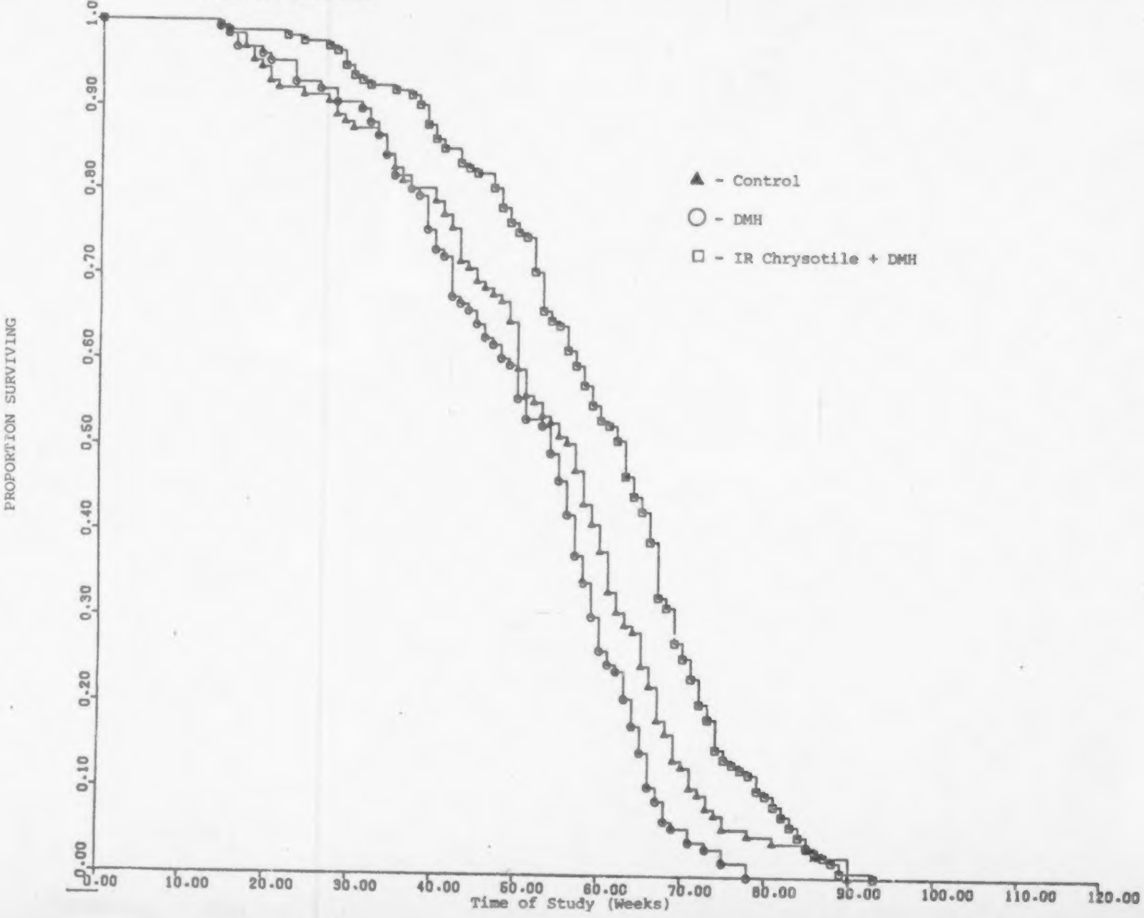


Table 6. Median Life Span of Hamsters in Oral Chrysotile Asbestos Study

| Group | Sex | Median Life Span (Weeks) |
|---|---|---|
| IR Chrysotile Control | M | 83 |
| | F | 61 |
| IR Chrysotile | M | 86 |
| | F | 59 |
| SR Chrysotile Control | M | 77 |
| | F | 57 |
| SR Chrysotile | M | 87 |
| | F | 63[a] |
| DMH and IR Chrysotile Control | M | 82 |
| | F | 57 |
| DMH | M | 82 |
| | F | 54[b] |
| IR Chrysotile and DMH | M | 90 |
| | F | 63[a] |

[a] Significantly ($P<0.05$) improved overall survival relative to controls (life table analysis).

[b] Significantly ($P<0.05$) reduced overall survival relative to controls (life table analysis).

Table 7. Incidence of Primary Tumors in Male Hamster Control Groups

| | SR Chrysotile Control | IR Chrysotile Control | DMH and IR Chrysotile Control | Amosite Control |
|---|---|---|---|---|
| Animals with primary tumors | 21/115 (18) | 26/116 (22) | 27/119 (23) | 21/122 (17) |
| Skin or sub. tissue: All tumors | 0/115 (0) | 1/116 (1) | 1/119 (1) | 0/122 (0) |
| Lung or trachea: All tumors | 0/115 (0) | 0/116 (0) | 0/119 (0) | 0/120 (0) |
| Adrenal: Cortical adenoma | 7/115 (6) | 7/115 (6) | 3/117 (3) | 8/119 (7) |
| Cortical carcinoma | 3/115 (3) | 3/115 (3) | 4/117 (3) | 3/119 (3) |
| Pheochromocytoma | 2/115 (2) | 5/115 (4) | 3/117 (3) | 3/119 (3) |
| Other tumors | 0/115 (0) | 3/115 (3) | 2/117 (2) | 1/119 (1) |
| Pancreas: Islet cell adenoma | 2/111 (2) | 7/110 (6) | 8/110 (7) | 3/114 (3) |
| Islet cell carcinoma | 1/111 (1) | 0/110 (0) | 0/110 (0) | 0/114 (0) |
| Thyroid: C-cell adenoma | 3/109 (3) | 3/106 (3) | 0/107 (0) | 1/106 (1) |
| C-cell carcinoma | 1/109 (1) | 1/106 (1) | 0/107 (0) | 1/106 (1) |
| Other | 0/109 (0) | 0/106 (0) | 1/107 (1) | 0/106 (0) |
| Parathyroid: Adenoma | 0/72 (0) | 1/71 (1) | 1/64 (2) | 0/64 (0) |
| G.I. Tract: All tumors | 2/115 (2) | 1/116 (1) | 2/119 (2) | 1/122 (1) |
| Pituitary: All tumors | 0/84 (0) | 0/77 (0) | 0/80 (0) | 0/81 (0) |
| Kidney: All tumors | 0/115 (0) | 2/116 (2) | 1/119 (1) | 1/120 (1) |
| Liver: All tumors | 0/115 (0) | 0/116 (0) | 0/119 (0) | 0/120 (0) |
| Leukemia or malignant lymphoma | 2/115 (2) | 1/116 (1) | 4/119 (3) | 1/122 (1) |
| Hemangioma or Hemangiosarcoma | 0/115 (0) | 0/116 (0) | 3/119 (3) | 2/122 (2) |
| All other tumors | 1/115 (1) | 0/116 (0) | 3/119 (3) | 1/122 (1) |

( ) Percent.

Table 8. Incidence of Primary Tumors in Female Hamster Control Groups

| | Short Range Chrysotile Control | Intermediate Range Chrysotile Control | DMH & Intermediate Range Chrysotile Control | Amosite Control |
|---|---|---|---|---|
| Animals with primary tumors | 19/114 (17) | 17/119 (14) | 15/120 (12) | 15/119 (13) |
| Skin or sub tissue: All tumors | 0/114 (0) | 0/119 (0) | 0/120 (0) | 0/119 (0) |
| Lung or trachea: All tumors | 0/114 (0) | 0/119 (0) | 0/119 (0) | 0/119 (0) |
| Adrenal: Cortical adenoma | 4/112 (4) | 6/118 (5) | 3/120 (2) | 2/118 (2) |
| Cortical carcinoma | 0/112 (0) | 0/118 (0) | 0/120 (0) | 0/118 (0) |
| Pheochromocytoma | 0/112 (0) | 0/118 (0) | 0/120 (0) | 0/118 (0) |
| Other tumors | 0/112 (0) | 0/118 (0) | 0/120 (0) | 0/118 (0) |
| Pancreas: Islet cell adenoma | 2/109 (2) | 5/116 (4) | 5/116 (4) | 3/115 (3) |
| Islet cell carcinoma | 1/109 (1) | 0/116 (0) | 0/116 (0) | 0/115 (0) |
| Thyroid: C-cell adenoma | 2/107 (2) | 3/115 (3) | 0/112 (0) | 1/106 (1) |
| C-cell carcinoma | 0/107 (0) | 0/115 (0) | 1/112 (1) | 0/106 (0) |
| Other | 2/107 (2) | 0/115 (0) | 0/112 (0) | 0/106 (0) |
| Parathyroid: Adenoma | 3/68 (4) | 1/77 (1) | 1/74 (1) | 1/61 (1) |
| G.I. Tract: All tumors | 1/114 (1) | 2/119 (2) | 1/120 (1) | 1/119 (1) |
| Pituitary: All tumors | 0/77 (0) | 2/67 (3) | 0/62 (0) | 0/79 (0) |
| Kidney: All tumors | 0/114 (0) | 1/119 (1) | 0/120 (0) | 0/119 (0) |
| Liver: All tumors | 0/114 (0) | 0/119 (0) | 0/119 (0) | 0/118 (0) |
| Leukemia or malignant lymphoma | 2/114 (2) | 0/119 (0) | 3/120 (2) | 2/119 (2) |
| Hemangioma or Hemangiosarcoma | 0/114 (0) | 0/119 (0) | 1/120 (1) | 1/119 (1) |
| Uterus: All tumors | 3/113 (3) | 1/119 (1) | 2/120 (2) | 2/119 (2) |
| All other tumors | 3/114 (3) | 0/119 (0) | 1/120 (1) | 2/119 (2) |

( ) Percent.

Table 9. Incidence of Primary Tumors in Male Hamsters Administered Short Range Chrysotile

| | Pooled Controls | Short Range Chrysotile Controls | Short Range Chrysotile |
|---|---|---|---|
| Animals with primary tumors | 95/472 (20) | 21/115 (18) | 64/233 (27)+ |
| Skin or sub. tissue: All tumors | 2/472 (<1) | 0/115 (0) | 0/233 (0) |
| Lung or trachea: All tumors | 0/470 (0) | 0/115 (0) | 0/231 (0) |
| Adrenal: Cortical adenoma | 25/466 (5) | 7/115 (6) | 26/229 (11)* |
| Cortical carcinoma | 13/466 (3) | 3/115 (3) | 8/229 (3) |
| Pheochromocytoma | 13/466 (3) | 2/115 (2) | 4/229 (2) |
| Other tumors | 6/466 (1) | 0/115 (0) | 1/229 (<1) |
| Pancreas: Islet cell adenoma | 20/445 (4) | 2/111 (2) | 15/218 (7) |
| Islet cell carcinoma | 1/445 (<1) | 1/111 (1) | 0/218 (0) |
| Thyroid: C-cell adenoma | 7/428 (2) | 3/109 (3) | 3/207 (1) |
| C-cell carcinoma | 3/428 (1) | 1/109 (1) | 1/207 (<1) |
| Other | 1/428 (<1) | 0/109 (0) | 0/207 (0) |
| Parathyroid: Adenoma | 2/271 (1) | 0/72 (0) | 3/132 (2) |
| G.I. Tract: All tumors | 6/472 (1) | 2/115 (2) | 0/233 (0) |
| Pituitary: All tumors | 0/322 (0) | 0/84 (0) | 0/159 (0) |
| Kidney: All tumors | 4/470 (1) | 0/115 (0) | 3/232 (1) |
| Liver: All tumors | 0/470 (0) | 0/115 (0) | 0/232 (0) |
| Leukemia or malignant lymphoma | 8/472 (2) | 2/115 (2) | 3/233 (1) |
| Hemangioma or Hemangiosarcoma | 5/472 (1) | 0/115 (0) | 4/233 (2) |
| All other tumors | 5/472 (1) | 1/115 (1) | 3/233 (1) |

( ) Percent

* $P<0.05$ vs. pooled controls.

+ P = .152 (life table); P = .065 (incidental tumor test) and P = .019 (Fisher's exact test) vs. pooled controls.

Table 10. Incidence of Primary Tumors in Female Hamsters Administered Short Range Chrysotile

| | Pooled Controls | Short Range Chrysotile Controls | Short Range Chrysotile |
|---|---|---|---|
| Animals with primary tumors | 66/472 (14) | 19/114 (17) | 28/228 (12) |
| Skin or sub tissue: All tumors | 0/472 (0) | 0/114 (0) | 3/228 (1) |
| Lung or Trachea: All tumors | 0/471 (0) | 0/114 (0) | 0/228 (0) |
| Adrenal: Cortical adenoma | 15/468 (3) | 4/112 (4) | 8/226 (4) |
| Cortical carcinoma | 0/468 (0) | 0/112 (0) | 0/226 (0) |
| Pheochromocytoma | 0/468 (0) | 0/112 (0) | 3/226 (1) |
| Other tumors | 0/468 (0) | 0/112 (0) | 1/226 (<1) |
| Pancreas: Islet cell adenoma | 15/456 (3) | 2/109 (2) | 2/217 (1)* |
| Islet cell carcinoma | 1/456 (<1) | 1/109 (1) | 0/217 (0) |
| Thyroid: C-cell adenoma | 6/440 (1) | 2/107 (2) | 0/214 (0) |
| C-cell carcinoma | 1/440 (<1) | 0/107 (0) | 0/214 (0) |
| Other | 2/440 (<1) | 2/107 (2) | 0/214 (0) |
| Parathyroid: Adenoma | 6/280 (2) | 3/68 (4) | 3/139 (2) |
| G.I. Tract: All tumors | 5/472 (1) | 1/114 (1) | 1/228 (<1) |
| Pituitary: All tumors | 2/285 (1) | 0/77 (0) | 1/132 (1) |
| Kidney: All tumors | 1/472 (<1) | 0/114 (0) | 0/228 (0) |
| Liver: All tumors | 0/472 (0) | 0/114 (0) | 0/228 (0) |
| Leukemia or malignant lymphoma | 7/472 (1) | 2/114 (2) | 2/228 (1) |
| Hemangioma or Hemangiosarcoma | 2/472 (<1) | 0/114 (0) | 1/228 (<1) |
| Uterus: All tumors | 8/471 (2) | 3/113 (3) | 5/226 (2) |
| All other tumors | 6/472 (1) | 3/114 (3) | 3/228 (1) |

( ) Percent.

* $P < 0.05$ decrease relative to pooled controls (life table and incidental tumor test).

Table 11. Incidence of Primary Tumors in Male Hamsters Administered Intermediate Range Chrysotile

| | Pooled Controls | Intermediate Range Chrysotile Controls | Intermediate Range Chrysotile |
|---|---|---|---|
| Animals with primary tumors | 95/472 (20) | 26/116 (22) | 78/245 (32)$^{++*}$ |
| Skin or sub. tissue: All tumors | 2/472 (<1) | 1/116 (1) | 0/245 (0) |
| Lung or trachea: All tumors | 0/470 (0) | 0/116 (0) | 1/244 (<1) |
| Adrenal: Cortical adenoma | 25/466 (5) | 7/115 (6) | 24/244 (10)$^{+}$ |
| Cortical carcinoma | 13/466 (3) | 3/115 (3) | 7/244 (3) |
| Pheochromocytoma | 13/466 (3) | 5/115 (4) | 11/244 (5) |
| Other tumors | 6/466 (1) | 3/115 (3) | 1/244 (<1) |
| Pancreas: Islet cell adenoma | 20/445 (4) | 7/110 (6) | 15/226 (7) |
| Islet cell carcinoma | 1/445 (<1) | 0/110 (0) | 1/226 (<1) |
| Thyroid: C-cell adenoma | 7/428 (2) | 3/106 (3) | 5/216 (2) |
| C-cell carcinoma | 3/428 (1) | 1/106 (1) | 4/216 (2) |
| Other | 1/428 (<1) | 0/106 (0) | 1/216 (<1) |
| Parathyroid: Adenoma | 2/271 (1) | 1/71 (1) | 4/138 (3) |
| G.I. Tract: All tumors | 6/472 (1) | 1/116 (1) | 3/245 (1) |
| Pituitary: All tumors | 0/322 (0) | 0/77 (0) | 0/182 (0) |
| Kidney: All tumors | 4/470 (1) | 2/116 (2) | 1/245 (<1) |
| Liver: All tumors | 0/470 (0) | 0/116 (0) | 0/244 (0) |
| Leukemia or malignant lympnoma | 8/472 (2) | 1/116 (1) | 10/245 (4) |
| Hemangioma or Hemangiosarcoma | 5/472 (1) | 0/116 (0) | 1/242 (<1) |
| All other tumors | 5/472 (1) | 0/116 (0) | 2/245 (1) |

( ) Percent

* $P<0.05$ vs. Intermediate Range Chrysotile controls.

+ $P<0.05$ vs. pooled controls.

++ $P<0.01$ vs. pooled controls.

Table 12. Incidence of Primary Tumors in Female Hamsters Administered Intermediate Range Chrysotile

| | Pooled Controls | Intermediate Range Chrysotile Controls | Intermediate Range Chrysotile |
|---|---|---|---|
| Animals with primary tumors | 66/472 (14) | 17/119 (14) | 39/244 (16) |
| Skin or sub tissue: All tumors | 0/472 (0) | 0/119 (0) | 2/244 (1) |
| Lung or trachea: All tumors | 0/471 (0) | 0/119 (0) | 0/243 (0) |
| Adrenal: Cortical adenoma | 15/468 (3) | 6/118 (5) | 18/234 (8)* |
| Cortical carcinoma | 0/468 (0) | 0/118 (0) | 1/234 (<1) |
| Pheochromocytoma | 0/468 (0) | 0/118 (0) | 1/234 (<1) |
| Other tumors | 0/468 (0) | 0/118 (0) | 0/234 (0) |
| Pancreas: Islet cell adenoma | 15/456 (3) | 5/116 (4) | 4/236 (2) |
| Islet cell carcinoma | 1/456 (<1) | 0/116 (0) | 0/236 (0) |
| Thyroid: C-cell adenoma | 6/440 (1) | 3/115 (3) | 2/223 (1) |
| C-cell carcinoma | 1/440 (<1) | 0/115 (0) | 0/223 (0) |
| Other | 2/440 (<1) | 0/115 (0) | 1/223 (<1) |
| Parathyroid: Adenoma | 6/280 (2) | 1/77 (1) | 1/148 (1) |
| G.I. Tract: All tumors | 5/472 (1) | 2/119 (2) | 1/244 (<1) |
| Pituitary: All tumors | 2/285 (1) | 2/67 (3) | 2/164 (1) |
| Kidney: All tumors | 1/472 (<1) | 1/119 (1) | 0/243 (0) |
| Liver: All tumors | 0/472 (0) | 0/119 (0) | 0/243 (0) |
| Leukemia or malignant lymphoma | 7/472 (1) | 0/119 (0) | 2/244 (1) |
| Hemangioma or Hemangiosarcoma | 2/472 (<1) | 0/119 (0) | 1/244 (<1) |
| Uterus: All tumors | 8/471 (2) | 1/119 (1) | 7/240 (3) |
| All other tumors | 6/472 (1) | 0/119 (0) | 2/244 (1) |

*$P<0.05$ vs. pooled controls.

( ) Percent.

Table 13. Incidence of Primary Tumors in Male Hamsters Administered 1,2-Dimethylhydrazine (DMH) or Intermediate Range Chrysotile and DMH

| | Pooled Controls | DMH & Intermediate Range Chrysotile Controls | DMH | DMH & Intermediate Range Chrysotile |
|---|---|---|---|---|
| Animals with primary tumors | 95/472 (20) | 27/119 (23) | 29/127 (23) | 51/173 (29)+ |
| Skin or sub tissue: All tumors | 2/472 (<1) | 1/119 (1) | 0/127 (0) | 1/173 (1) |
| Lung or trachea: All tumors | 0/470 (0) | 0/119 (0) | 0/126 (0) | 0/173 (0) |
| Adrenal: Cortical adenoma | 25/466 (5) | 3/117 (3) | 3/127 (2) | 8/171 (5) |
| Cortical carcinoma | 13/466 (3) | 4/117 (4) | 2/127 (2) | 7/171 (4) |
| Pheochromocytoma | 13/466 (3) | 3/117 (3) | 4/127 (3) | 6/171 (4) |
| Other tumors | 6/466 (1) | 2/117 (2) | 0/127 (0) | 1/171 (1) |
| Pancreas: Islet cell adenoma | 20/445 (4) | 8/110 (7) | 6/114 (5) | 10/167 (6) |
| Islet cell carcinoma | 1/445 (<1) | 0/110 (0) | 0/114 (0) | 1/167 (1) |
| Thyroid: C-cell adenoma | 7/428 (2) | 0/107 (0) | 2/118 (2) | 3/163 (2) |
| C-cell carcinoma | 3/428 (1) | 0/107 (0) | 0/118 (0) | 1/163 (1) |
| Other | 1/428 (<1) | 1/107 (1) | 0/118 (0) | 0/163 (0) |
| Parathyroid: Adenoma | 2/271 (1) | 1/64 (2) | 0/81 (0) | 2/118 (2) |
| G.I. Tract: All tumors | 6/472 (1) | 2/119 (2) | 3/127 (2) | 4/173 (2) |
| Pituitary: All tumors | 0/322 (0) | 0/80 (0) | 1/87 (1) | 2/123 (2) |
| Kidney: All tumors | 4/470 (1) | 1/119 (1) | 0/127 (0) | 0/173 (0) |
| Liver: All tumors | 0/470 (0) | 0/119 (0) | 2/127 (2) | 1/173 (1) |
| Leukemia or malignant lymphoma | 8/472 (2) | 4/119 (4) | 7/127 (6)* | 8/173 (5) |
| Hemangioma or Hemangiosarcoma | 5/472 (1) | 3/119 (3) | 2/127 (2) | 2/173 (1) |
| All other tumors | 5/472 (1) | 3/119 (3) | 1/127 (1) | 4/173 (2) |

( ) Percent

+ P = 0.257 (life table); P = 0.038 (incidental tumor test); P = 0.009 (Fisher's exact test) vs. pooled controls.

* P<0.05 vs. pooled controls.

Table 14. Incidence of Primary Tumors in Female Hamsters Administered 1,2-Dimethylhydrazine (DMH) or Intermediate Range Chrysotile and DMH

| | Pooled Controls | DMH & Intermediate Range Chrysotile Control | DMH | DMH & Intermediate Range Chrysotile |
|---|---|---|---|---|
| Animals with primary tumors | 66/472 (14) | 15/120 (12) | 15/122 (12) | 19/161 (12) |
| Skin or sub tissue: All tumors | 0/472 (0) | 0/120 (0) | 1/122 (1) | 0/161 (0) |
| Lung or trachea: All tumors | 0/471 (0) | 0/119 (0) | 0/122 (0) | 1/160 (1) |
| Adrenal: Cortical adenoma | 15/468 (3) | 3/120 (2) | 2/120 (2) | 6/158 (4) |
| Cortical carcinoma | 0/468 (0) | 0/120 (0) | 0/120 (0) | 2/158 (1) |
| Pheochromocytoma | 0/468 (0) | 0/120 (0) | 0/120 (0) | 0/158 (0) |
| Other tumors | 0/468 (0) | 0/120 (0) | 0/120 (0) | 0/158 (0) |
| Pancreas: Islet cell adenoma | 15/456 (3) | 5/116 (4) | 2/119 (2) | 4/149 (3) |
| Islet cell carcinoma | 1/456 (<1) | 0/116 (0) | 0/119 (0) | 0/149 (0) |
| Thyroid: C-cell adenoma | 6/440 (1) | 0/112 (0) | 0/108 (0) | 0/141 (0) |
| C-cell carcinoma | 1/440 (<1) | 1/112 (1) | 0/108 (0) | 0/141 (0) |
| Other | 2/440 (<1) | 0/112 (0) | 0/108 (0) | 0/141 (0) |
| Parathyroid: Adenoma | 6/280 (2) | 1/74 (1) | 2/57 (4) | 0/91 (0) |
| G.I. Tract: All tumors | 5/472 (1) | 1/120 (1) | 2/122 (2) | 0/161 (0) |
| Pituitary: All tumors | 2/285 (1) | 0/62 (0) | 0/59 (0) | 0/109 (0) |
| Kidney: All tumors | 1/472 (<1) | 0/120 (0) | 0/122 (0) | 0/161 (0) |
| Liver: All tumors | 0/472 (0) | 0/119 (0) | 0/121 (0) | 0/161 (0) |
| Leukemia or malignant lymphoma | 7/472 (1) | 3/120 (2) | 2/122 (2) | 3/161 (2) |
| Hemangioma or Hemangiosarcoma | 2/472 (<1) | 1/120 (1) | 0/122 (0) | 1/161 (1) |
| Uterus: All tumors | 8/471 (2) | 2/120 (2) | 2/116 (2) | 2/156 (1) |
| All other tumors | 6/472 (1) | 1/120 (1) | 2/122 (2) | 2/161 (1) |

( ) Percent.

Table 15. Incidence of Gastrointestinal Tract Tumors
Observed in Chrysotile Asbestos Study

| | Pooled Controls | | Short Range Chrysotile | | Intermediate Range Chrysotile | | DMH | | DMH & Intermediate Range Chrysotile | |
|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | M | F |
| Stomach (no. examined) | (464) | (468) | (222) | (224) | (244) | (242) | (127) | (118) | (170) | (160) |
| Squamous-cell papilloma | 3 | | | | 1 | | | | 2 | |
| Carcinoma in-situ | | | | | 1 | | | | | |
| Papillary adenoma | | | | | 1 | 1[a] | | | | |
| Small Intestine (no. examined) | (467) | (469) | (226) | (227) | (244) | (244) | (127) | (120) | (170) | (159) |
| Adenoma | 1 | | | | | | | | | |
| Adenomacarcinoma | 1 | | | | | | | | | |
| Large intestine (no. examined) | (464) | (468) | (222) | (226) | (241) | (243) | (126) | (118) | (170) | (159) |
| Papilloma | | | | | | | | | 1 | |
| Adenoma | | 1[a] | | | | | 1 | | | |
| Papillary adenoma | | | | | | | 1 | | | |
| Adenocarcinoma | | 1 | | 1 | | | | | | |
| Lipoma | | 1 | | | | | | | | |
| Adenomatous Polyp | | | | | | | | 1[a] | | |
| Rectum (no. examined) | (472) | (472) | (233) | (228) | (245) | (244) | (127) | (122) | (173) | (161) |
| Adenoma | 1 | 1[a] | | | | | | | | |
| Papillary adenoma | | | | | | | | | 1[a] | |
| Fibrosarcoma | | | | | | | 1 | | | |
| Squamous cell carcinoma | | | | | | | | 1 | | |
| Fibroma | | 1 | | | | | | | | |

[a] The PWG did not diagnose these lesions as tumors.

The only group to show a significant ($p<0.05$) increase in overall primary tumors was the male IR chrysotile group. This increase was due primarily to adrenal tumors. Male hamsters receiving SR chrysotile or DMH and IR chrysotile also showed an elevated incidence of primary tumors relative to pooled controls. However, when survival differences were taken into account by a life table analysis, these differences were not statistically significant (Tables 9 and 13). Female chrysotile groups showed little evidence of an increased incidence of primary tumors relative to temporal or pooled controls.

The diagnoses of the NTP Pathology Working Group differed with the original pathologists interpretation as shown in Table 16. The major diagnostic difference was in regard to whether certain adrenal tumors originated in the medulla or cortex. As shown in Table 16, the PWG diagnoses reduced the significance of adrenal cortical tumors in the male SR chrysotile group but did not materially alter the results in other groups. The criteria used by the NTP/PWG for the diagnosis of adrenal tumors were those reported by Homburger and Russfield, 1970; Matsuyama and Suzuki, 1970; and Murthy and Russfield, 1966. The criteria were as follows:

Adrenal Cortex:

1. Focal hyperplasia - Proliferation of normal appearing cells resembling those of the zona fasciculata. The cells were of uniform size and morphology and mitotic figures were not observed. These lesions were invariably observed in adrenals showing severe amyloidosis. Another type of hyperplastic lesion encountered was the presence of extracapsular nodules of normal appearing cortical tissue. These were completely encased in a connective tissue capsule.

2. Cortical adenoma - There were two types observed. One type was composed of cells resembling the zona fasciculata which were somewhat pheomorphic and were compressing the adjacent parenchyma. The second type contained similar cells but admixed between them were spindle shaped cells which resembled fibroblasts. Mitotic figures were rare. The border of both types of adenomas was well defined but no capsule was evident.
3. Neurolemoma - This tumor was composed of delicate spindloid cells arranged in parallel palasades. It was well circumscribed but nonencapsulated. No mitotic figures were observed. This type of tumor was of much lower incidence than the previously described adenomas.
4. Cortical carcinoma - Carcinomas were composed of cells resembling both types of the above described adenomas. They were differentiated from adenomas based on pleomorphism, nuclear atypia, increased numbers of mititoc figures and invasive growth through the capsule and/or into adjacent blood vessels. Areas of necrosis and hemorrhage were common.

Adrenal Medulla:

1. Hyperplasia - This lesion was characterized by proliferation of normal appearing cells, although an increase in basophilia was sometimes noted. The normal architecture was preserved and the lesion was usually diffuse in nature.
2. Pheochromocytoma - This lesion consisted of a focal nodular proliferation of fairly normal appearing uniform cells although they were often smaller than normal. Mitotic figures were not observed. Growth was by expansion. The borders were distinct and there appeared to be a delicate capsule.
3. Malignant Pheochromocytoma - The major distinguishing characteristics of this neoplasm were nuclear atypia along with invasive growth.

Table 16. Comparison of Adrenal Tumor Incidence as Determined by Original Pathologist (OP) and the NTP Pathology Working Group (PWG)

| | Pooled Controls | SR Chrysotile Controls | SR Chrysotile | IR Chrysotile Controls | IR Chrysotile | DMH & IR Chrysotile Controls | DMH | DMH & IR Chrysotile |
|---|---|---|---|---|---|---|---|---|
| Males (OP) | | | | | | | | |
| Cortical adenoma | 25/466 (5) | 7/115 (6) | 26/229 (11)$^*$ | 7/115 (6) | 24/244 (10)$^*$ | 3/117 (3) | 3/127 (2) | 8/171 (5) |
| Cortical carcinoma | 13/466 (3) | 3/115 (3) | 8/229 (3) | 3/115 (3) | 7/244 (3) | 4/117 (4) | 2/127 (2) | 7/171 (4) |
| Pheochromocytoma | 13/466 (3) | 2/115 (2) | 4/229 (2) | 5/115 (4) | 11/244 (5) | 3/117 (3) | 4/127 (3) | 6/171 (4) |
| Other | 6/466 (1) | 0/115 (0) | 1/229 (<1) | 3/115 (3) | 1/244 (<1) | 2/117 (2) | 0/127 (0) | 1/171 (1) |
| Males (PWG) | | | | | | | | |
| Cortical adenoma | 31/466 (7) | 7/115 (6) | 26/229 (11) | 9/115 (8) | 29/244 (12)$^*$ | 6/117 (5) | 5/127 (4) | 11/171 (6) |
| Cortical carcinoma | 14/466 (3) | 3/115 (3) | 9/229 (4) | 3/115 (3) | 7/244 (3) | 5/117 (4) | 4/127 (3) | 8/171 (5) |
| Pheochromocytoma | 7/466 (2) | 2/115 (2) | 2/229 (1) | 4/115 (3) | 7/244 (3) | 0/117 (0) | 2/127 (2) | 1/171 (1) |
| Other | 2/466 (<1) | 0/115 (0) | 1/229 (<1) | 1/115 (1) | 1/244 (<1) | 1/117 (1) | 0/127 (0) | 1/171 (1) |
| Females (OP) | | | | | | | | |
| Cortical adenoma | 15/468 (3) | 4/112 (4) | 8/226 (4) | 6/118 (5) | 18/234 (8)$^*$ | 3/120 (2) | 2/120 (2) | 6/158 (4) |
| Cortical carcinoma | 0/468 (0) | 0/112 (0) | 0/226 (0) | 0/118 (0) | 1/234 (<1) | 0/120 (0) | 0/120 (0) | 2/158 (1) |
| Pheochromocytoma | 0/468 (0) | 0/112 (0) | 3/226 (1) | 0/118 (0) | 1/234 (<1) | 0/120 (0) | 0/120 (0) | 0/158 (0) |
| Other | 0/468 (0) | 0/112 (0) | 1/226 (<1) | 0/118 (0) | 0/234 (0) | 0/120 (0) | 0/120 (0) | 0/158 (0) |
| Females (PWG) | | | | | | | | |
| Cortical adenoma | 19/468 (4) | 4/112 (4) | 9/226 (4) | 7/118 (6) | 23/234 (10)$^*$ | 4/120 (3) | 2/120 (2) | 7/158 (4) |
| Cortical carcinoma | 0/468 (0) | 0/112 (0) | 0/226 (0) | 0/118 (0) | 1/234 (<1) | 0/120 (0) | 0/120 (0) | 2/158 (1) |
| Pheochromocytoma | 0/468 (0) | 0/112 (0) | 2/226 (1) | 0/118 (0) | 1/234 (<1) | 0/120 (0) | 0/120 (0) | 0/158 (0) |
| Other | 0/468 (0) | 0/112 (0) | 1/226 (<1) | 0/118 (0) | 0/234 (0) | 0/120 (0) | 0/120 (0) | 0/158 (0) |

$^*$ $P<0.05$ relative to pooled controls.

The major difference in terminology between the original pathologist and the NTP/PWG was in regard to the adrenal tumors (benign and malignant) composed of a mixture of spindle shaped cells and eosinophilic hepatoid-like cells. The original pathologist diagnosed these as pheochromocytomas or malignant pheochromocytomas while the PWG called them cortical adenomas or carcinomas.

While this study was not designed to evaluate non-neoplastic disease, noteworthy lesions were observed although none were apparently treatment related and are consistent with what is normally found in aging hamsters. It was the pathologists' opinion that the most important lesion, responsible for many deaths, was generalized amyloidosis. The kidneys were particularly affected by diffuse accumulation of amyloid which replaced glomeruli and infiltrated tubular interstitium to the point where the normal cortical architecture was obliterated. Other organs which showed significant accumulations of amyloid were adrenal gland, liver, spleen and the epithelium of the small intestine. Amyloid within the walls of blood vessels was observed in many tissues.

Many of the livers were cirrhotic infiltrated with amyloid, and contained large cystic structures filled with a lightly staining proteinaceous fluid. These structures were interpreted as cystic bile ducts and are consistent with what others have termed "retention cysts". At times these cysts were so large and/or numerous that less than half of the livers remained.

Other non-neoplastic lesions that were observed in greater than 5% of the hamsters in any of the experimental groups are as follows:

1. Skin - chronic dermatitis
2. Lung - interstitial pneumonitis
3. Spleen - lymphoid atrophy
4. Lymph node - hyperplasia
5. Heart - atrial thrombosis
6. Gall Bladder - edema and calculi

7. Stomach (nonglandular) - hyperkeratosis or acanthosis
8. Colon - intussusception, inflammation
9. Urinary bladder - chronic inflammation, hyperplasia
10. Adrenal gland - cortical and medullary hyperplasia
11. Thyroid gland - follicular atrophy
12. Pituitary gland - degeneration
13. Ovary - atrophy
14. Uterus - inflammation, endometrial hyperplasia
15. Vagina - acute inflammation, squamous metaplasia.

None of these lesions was treatment related.

## IV. SUMMARY, COMMENTS AND CONCLUSION

The clinicopathologic results in this study showed that the chronic ingestion of 1% chrysotile (SR and IR) asbestos in the diet did not cause any adverse effect on body weight gain and survival. In fact, both weight gain and survival seemed to be enhanced. An explanation for these observations is not apparent.

The major organ which showed a statistically significant ($p<0.05$) increased rate of neoplasia was the adrenal cortex in male and female hamsters exposed to IR chrysotile and males exposed to SR chrysotile when compared to pooled controls. However, statistical significance was not present when these groups were compared to their temporal controls. Also, the increased incidence in SR males was not statistically significant using the pathology data of the NTP/PWG. The increase in body weight may have been a factor in adrenal cortical tumorigenesis but this is speculative. It is difficult to envision how oral asbestos, even though it is known to be absorbed through the gastrointestinal tract (Cook and Olson, 1979), could cause an increased tumor rate in the adrenal cortex without causing similar increases in tumors in other abdominal organs and tissues, i.e., gastrointestinal tract and peritoneum. For these reasons the biologic importance of adrenal tumors in this study is doubtful. The overall increase in total primary tumors in male IR chrysotile hamsters can be explained primarily on the basis of an increased incidence of adrenal tumors in this group. The enhanced survival of animals in the chrysotile groups also contributed to the elevated incidence of primary tumors observed in these groups compared to controls.

The only other instance of an increased rate of neoplasia was a significant ($P<0.05$) increase in leukemia or malignant lymphoma in male hamsters exposed to DMH when compared to pooled controls. Again, statistical significance was no longer evident when this group was compared to its temporal control group. This finding also loses importance because it was not observed in the DMH plus IR chrysotile group.

Other such studies involving the long term ingestion of asbestos are few. Donham, et al., (1980) reported equivocal results in F344 rats which were fed a diet containing 10% chrysotile for their lifetime. While they did not observe a statistically significant ($p<0.05$) increase in the number of tumors in exposed animals, the authors believed that there was a trend toward increased colon lesions in general, evidence of penetration of asbestos into the colonic mucosa, possible cytotoxicity to colonic tissues and suggested a possible relationship to peritoneal mesothelioma. Another equivocal study is that reported by Gibel, et al., (1976) who described an increase in malignant tumors in the lung, kidney, liver and reticuloendothelial system but no increase in intestinal neoplasia in Wistar rats fed asbestos filter material (20 mg/day) for a period of 8-14 months. Cunningham, et al., (1977) reported 2 studies in Wistar male rats using 1% chrysotile in the diet; one study of 24 months and one of 30 months. No intestinal tumors were found in the control rats. Negative results were reported by Gross, et al., (1974), who fed rats a diet containing 5% chrysotile asbestos for a period of 21 months with no evidence of intestinal neoplasia.

The only oral asbestos study in hamsters was reported by Smith, et al., (1980). They exposed groups of 30 male and female hamsters via drinking water for lifetime to amosite asbestos, mine tailings, beach rock and Lake Superior drinking water. They did not observe adverse effects on body weight or survival time in any of the groups. A peritoneal mesothelioma, one pulmonary carcinoma, and two early squamous cell carcinomas of the nonglandular stomach were found in the hamsters exposed to amosite but the incidence was not statistically significant ($p>0.05$). Their other studies were considered negative. They concluded that the study was essentially negative.

Except for the studies of Donham, et al., (1980) and Smith, et al., (1980), the other studies were conducted with relatively small numbers of animals. Also some were conducted for an insufficient period of time to adequately test the carcinogenic potential of ingested asbestos.

The results of the combination study (IR chrysotile plus DMH) also did not yield a significant increase in tumors above the background level observed in the DMH group ~~alone or~~ in the untreated control group. The DMH failed to yield a background level of intestinal tumors high enough to provide a valid test of the cocarcinogenic potential of chrysotile asbestos. For this reason the cocarcinogenic potential of oral asbestos should be considered untested. However, the DMH plus chrysotile provides an additional IR chrysotile group for comparative purposes.

It is not clear why the DMH treated group of hamsters failed to show an increased incidence of intestinal neoplasia. The pilot study suggested that this dose of DMH should have caused an incidence of around 15%. DMH solutions rapidly decompose at room temperature, particularly if not properly buffered. It is probably the DMH was not properly buffered explaining the lack of its carcinogenic effect.

The only long term study designed to determine the cocarcinogenic potential of asbestos was reported by Ward, et al., (1980). They exposed 6 week old male F344 rats 3 times per week for 10 weeks to 1 mg amosite asbestos in saline via gavage. Once per week during this same period half of the rats received subcutaneous injections of 7.4 mg/kg azoxymethane (AOM), a known intestinal carcinogen in animals. The rats were allowed to live out their life span or until they reached 94-95 weeks of age at which time they were killed. They reported an intestinal tumor incidence of 66.7% in AOM alone, 77.1% for amosite plus AOM and 32.6% for amosite alone. The authors concluded that while amosite did not significantly add to the incidence of AOM-induced intestinal neoplasia, amosite alone caused a relatively high rate of intestinal neoplasia. However, there was no untreated control group to compare to the treated groups. These results should also be viewed with some suspicion because the authors also reported a 14% incidence of Zymbal gland tumors in the rats exposed to amosite alone. The historical rate of Zymbal gland tumors in the Bioassay Program is 0.34%, indicating that this is an extremely rare spontaneous tumor. However, AOM is known to induce Zymbal gland tumors with a single dose of 5.1 mg/kg in male F344 rats producing a 14% incidence of tumors in this organ (Ward, 1975); in this study 5.1 mg/kg AOM also caused a 24% incidence of intestinal neoplasia. An appropriate explanation for the high incidence of Zymbal gland tumors in the amosite group would be that they were inadvertently exposed to AOM. If this occurred they would also be expected to show a high incidence of intestinal neoplasia.

It should be emphasized that this investigation of the carcinogenic and cocarcinogenic potential of ingested asbestos is a two animal species effort by the National Toxicology Program. While the results in the hamster appear to be negative, a larger bioassay involving more types of asbestos but using essentially the same protocol (1% diet) in rats is currently being evaluated.

The concurrent study (NTP, 1981) using 1% amosite in the diet of hamsters did not show any increase in tumor incidence compared to pooled or temporal control groups.

CONCLUSION - Under the conditions of this bioassay the ingestion of short range chrysotile and intermediate range chrysotile was not carcinogenic in male and female Syrian golden hamsters. While there were significant in-creases in the rates of adrenal cortical adenomas in male and female hamsters exposed to IR chrysotile compared to pooled control groups, they were no longer significant when contrasted to the temporal control groups. Additionally, the biologic importance of adrenal tumors in the absence of target organ neoplasia is questionable. The combination studies using IR chrysotile and 1,2,dimethylhydrazine were considered inadequate because of a lack of an increase in intestinal neoplasia in the DMH group.

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## APPENDIX A

## CALCULATED VALUES OF SHORT RANGE CHRYSOTILE ASBESTOS IN INDIVIDUAL LOTS OF FEED AS DETERMINED BY MAGNESIUM CONTENT

| Feed Preparation Data | Calculated Asbestos Content (%) |
|---|---|
| 1-77 | 1.054 |
| 5-77 | .991 |
| 6-77 | 1.14 |
| 6-77 | .897 |
| 8-77 | .885 |
| 10-77 | .959 |
| 1-78 | 1.00 |
| 2-78 | .94 |
|  | .91 |
| 4-78 | 1.09 |
| 6-78 | .93 |
| 8-78 | .86 |
| 8-78 | .93 |
| 10-78 | .89 |
| 12-78 | .80 |
| 3-79 | .93 |
|  | 1.06 |
| 5-79 | 1.07 |
| 6-79 | 1.06 |
| 7-79 | .87 |
| 10-79 | .97 |
| 11-79 | .91 |
| 1-80 | .93 |
| 2-80 | .86 |
| 4-80 | .90 |
| 6-80 | 1.19 |

APPENDIX A (continued)

| Feed Preparation Data | Calculated Asbestos Content (%) |
|---|---|
| 1-77 | 1.05 |
| 3-77 | .954 |
| 5-77 | .856 |
| 6-77 | 1.064 |
| 8-77 | .942 |
| | .885 |
| 10-77 | .85 |
| | .887 |
| 1-78 | 1.02 |
| | 1.08 |
| 2-78 | 1.02 |
| | 1.03 |
| | 1.02 |
| 4-78 | 1.09 |
| | 1.10 |
| 6-78 | 1.02 |
| | .99 |
| 8-78 | .93 |
| | .91 |
| 10-78 | .91 |
| | .90 |
| | .92 |
| 12-78 | .85 |
| | .91 |
| 3-79 | 1.09 |
| | 1.04 |
| | 1.02 |
| | 1.11 |
| 6-79 | 1.00 |
| 7-79 | .95 |
| | .88 |

APPENDIX A (continued)

| Feed Preparation Data | Calculated Asbestos Content (%) |
|---|---|
| 10-79 | .99 |
| | 1.00 |
| 11-79 | 1.15 |
| | 1.02 |
| 1-80 | .99 |
| | 1.07 |
| 2-80 | .94 |
| | 1.01 |
| 4-80 | .91 |
| 6-80 | 1.14 |
| | 1.02 |

Note

These data represent unverified working values for all lots of feed used in the hamster and rat studies. Final data may show minor numerical variations. A complete description of the atomic absorption methodology utilized and these analyses will be described in the final report.

## Appendix B1

## Disease Status of Hamsters Exposed to Short Range Chrysotile Asbestos

| | Control | | Asbestos (Short Range Chrysotile) | |
|---|---|---|---|---|
| Organism | Mother | Offspring | Mother | Offspring |
| *Mycoplasma* spp. | - | - | - | - |
| *Corynebacterium kutscheri* | - | - | - | - |
| *Salmonella* spp. | - | - | - | - |
| *Streptobacillus moniliformis* | - | - | - | - |
| *Streptococcus pneumoniae* | - | - | - | - |
| *Haemobartonella* spp. | - | - | - | - |
| *Encephalitazoon* spp. | - | - | - | - |
| *Aspicularis tetraptera* | - | - | - | - |
| *Syphacia obvelata* | - | - | - | - |
| *Taxoplasma gondii* | - | - | - | - |
| *Hymenolepsis diminuta* or *nana* | - | - | - | - |
| *Trichomonas* spp. | +++ | +++ | +++ | +++ |
| *Hexamita muris* | + | + | ++ | ++ |
| *Giardia muris* | ++ | ++ | + | + |
| *Lymphocytic choriomeningitis* | - | - | - | - |
| Mites | - | - | - | - |
| Fleas | - | - | - | - |
| Lice | - | - | - | - |

## Appendix B2

## Disease Status of Hamsters Exposed to Intermediate Range Chrysotile Asbestos

| | Control | | Asbestos (Intermediate Range Chrysotile) | |
|---|---|---|---|---|
| Organism | Mother | Offspring | Mother | Offspring |
| *Mycoplasma* spp. | - | - | - | - |
| *Corynebacterium kutscheri* | - | - | - | - |
| *Salmonella* spp. | - | - | - | - |
| *Streptobacillus moniliformis* | - | - | - | - |
| *Streptococcus pneumoniae* | - | - | - | - |
| *Haemobartonella* spp. | - | - | - | - |
| *Encephalitazoon* spp. | - | - | - | - |
| *Aspicularis tetraptera* | - | - | - | - |
| *Syphacia obvelata* | - | - | - | - |
| *Taxoplasma gondii* | - | - | - | - |
| *Hymenolepsis diminuta* or *nana* | - | - | - | - |
| *Trichomonas* spp. | +++ | +++ | +++ | +++ |
| *Hexamita muris* | + | + | ++ | ++ |
| *Giardia muris* | ++ | ++ | ++ | ++ |
| *Lymphocytic choriomeningitis* | - | - | - | - |
| Mites | - | - | - | - |
| Fleas | - | - | - | - |
| Lice | - | - | - | - |

## Appendix B3

### Disease Status of Hamsters Exposed to Intermediate Range Chrysotile and/or 1,2-Dimethylhydrazine

| | Control | | Treatment Groups<br>Intermediate Range<br>Chrysotile and/or DMH | |
|---|---|---|---|---|
| Organism | Mother | Offspring | Mother | Offspring |
| *Mycoplasma* spp. | - | - | - | - |
| *Corynebacterium kutscheri* | - | - | - | - |
| *Salmonella* spp. | - | - | - | - |
| *Streptobacillus moniliformis* | - | - | - | - |
| *Streptococcus pneumoniae* | - | - | - | - |
| *Haemobartonella* spp. | - | - | - | - |
| *Encephalitazoon* spp. | - | - | - | - |
| *Aspicularis tetraptera* | - | - | - | - |
| *Syphacia obvelata* | +++ | +++ | +++ | +++ |
| *Taxoplasma gondii* | - | - | - | - |
| *Hymenolepsis diminuta* or *nana* | - | - | - | - |
| *Trichomonas* spp. | +++ | +++ | +++ | +++ |
| *Hexamita muris* | ++ | ++ | ++ | ++ |
| *Giardia muris* | ++ | ++ | ++ | ++ |
| *Lymphocytic choriomeningitis* | - | - | - | - |
| Mites | - | - | - | - |
| Fleas | - | - | - | - |
| Lice | - | - | - | - |

## Appendix C

### Analysis of 1,2-Dimethylhydrazine Solutions

| Date prepared and used | Theoretical Concentration | Actual Concentration |
|---|---|---|
| 6-22-77 | 2.00 mg/ml | 2.004 ± 0.03 mg/ml |
| 7-6-77 | 2.00 mg/ml | 1.75 ± 0.04 mg/ml |
| 7-20-77 | 2.00 mg/ml | 2.51 ± 0.08 mg/ml |
| 8-3-77 | 2.00 mg/ml | 1.92 ± 0.06 mg/ml |
| 8-17-77 | 2.00 mg/ml | 1.68 ± 0.14 mg/ml |

The concentration of 1,2-dimethylhydrazine was determined by the pentacyanoamino ferrate colorimetric method. Quantity modifications were made to avoid the small and fraction milliliter quantities and to make possible the use of standard laboratory ware.

All solutions and solid reagents, in ure or storage were kept cold, at about ice temperatures in a cold refrigerator.

Reagents

1. Trisodium salt of pentacyanoamino ferrate; concentration 20 mg/ml or 2.0 g/100 ml dissolved in cold borate buffer.
2. Stock 1,2-DMH·2HCl solution; approximately 40 mg/200 ml; dissolved in cold acetate buffer; concentration, approximately 200 µg/ml.
3. 0.05 M Borate buffer:

   0.05 M $H_3BO_3$:3.08 g/l

   0.5 M $Na_3B_4O_7 \cdot 10\ H_2O$:19.06 g/l

   Add the salt to boric acid until pH 8.5.

4. 0.2 M acetate buffer

   0.2 M HOAc:11.4 ml conc glacial acetic acid per liter. Conc HOAc = 17.6 M

   0.2 M NaOAc:27.2 g/liter of $NaOAc \cdot 3H_2O$

   Add HOAc to the salt solution until pH 5.

5. 1-1 HCl solution.

6. Color development reagent (combined reagent) 12.0 ml (by pipette) of pentacyanoamino ferrate reagent diluted to 250 ml with cold borate buffer. Keep cold.

Equipment

Beckman spectrophotometer; 1 cm cells. Wavelength 536 nm.

Calibration Curve

No attempt was made to weigh 40.0 mg of the symmetrical DMH·2HCl because of its unstable nature at room temperature, and its tendency to pick up water. 40 mg or more were quickly weighed and immediately dissolved in cold acetate buffer and diluted to 200 ml in a volumetric flask.

To prepare the calibration curve 6 standard solutions were prepared from which 5 ml aliquots were taken to obtain each absorbance value.

| Std Solution | | If Stock is 210 μg/ml | |
|---|---|---|---|
| ml Stock | ml $OAc^-$ Buffer | Std Sol μg/ml | Calib Sol μg/ml |
| 4 | 36 | 21 | 3.44 |
| 8 | 32 | 42 | 6.88 |
| 10 | 30 | 52.5 | 8.61 |
| 12 | 28 | 63 | 10.3 |
| 16 | 24 | 84 | 13.8 |
| 20 | 20 | 105 | 17.2 |

The calibration curve points were obtained by 5 aliquots of 5 ml each diluted with 25 ml color reagent and 0.5 ml of 1-1 HCl solution or a total or

30.5 ml. Concentration in μg/ml for each of these calibration points are (5 ml X conc each std μg/ml) ÷ 30.5 ml and are included in the above table. The blank consists of 5 ml OAc$^-$ buffer with 25 ml indicator and 0.5 ml 1-1 acid.

The color develops and fades very rapidly, even when cold. To obtain reasonably consistent values for 5 aliquots the acid was measured with a fast pipette, a dry cuvette filled and read immediately. If any motion is delayed this is reflected in a bad reading. A typical calibration curve and copy of a data sheet are attached.

A new calibration curve was prepared for each sample analyzed. Because these curves were prepared from somewhat different concentrations (plotted according to ml) two curves prepared from two different concentrations and two weeks apart were converted to equivalent concentrations and plotted. The two curves were nearly identical.

### Sample Analysis

For sample analyses, samples were received as a solution; three separate aliquots were taken from the original sample. These were diluted to 50 ml; then a 10 ml sample was taken from each and diluted to 50 ml. From the latter 5 separate samples were taken, each diluted to 30.5 ml and the absorbance values averaged to determine the concentration. The 5 absorbance values were averaged to give the concentration of each aliquot.